STUDIES IN THE PHYSIOLOGY OF THE FUNGI

XV. GERMINATION OF THE SPORES OF CERTAIN FUNGI IN RELATION TO HYDROGEN–ION CONCENTRATION[1]

ROBERT W. WEBB

Formerly Rufus J. Lackland Research Fellow in the Henry Shaw School of Botany of Washington University.

INTRODUCTION

The effect of the hydrogen- and the hydroxyl-ion concentration in a mannite medium upon germination of the spores of certain fungi, *Aspergillus niger*, *Penicillium cyclopium*, *Botrytis cinerea*, *Fusarium* sp., and *Lenzites saepiaria*, and the range within which their most favorable germination occurs have been presented in a recent paper (Webb, '19). Very striking data were obtained from this preliminary study, the features of particular interest being (1) the importance of active acidity in germination and (2) the relatively low percentage of germination under conditions of active alkalinity. It was found, in general, that the majority of the fungi employed showed maximum germination of the spores with relatively high acidity, namely, P_H 3.1, and further, that none of the fungi suffered inhibition of germination on the acid side of neutrality until a hydrogen-ion concentration greater than that of P_H 2.8 was passed. Germination quantities in most cases decreased, though not necessarily proportionally, with decrease in hydrogen-ion concentration from the optimum concentration previously mentioned. Certain forms, however, such as *Penicillium cyclopium* and *Fusarium* sp., exhibited secondary maxima at or near neutrality.

The medium employed in that investigation contained mannite as the sole nutrient, and its hydrogen- and hydroxyl-ion concentrations were adjusted by means of equal additions of orthophosphoric acid and successively increasing additions of sodium hydroxide. Logical questions which naturally arise at this point are: Is the effect of the hydrogen- and hydroxyl-ion concentration upon the rate of germination of the spores of certain fungi the same in different types of media? Is the range within which the most favorable germination occurs the same for different types of media? What relation does nutrition bear, if any, to the toxicity of the hydrogen- and hydroxyl-ion concentration during germination? And, if certain differences in effect exist, what are the explanations of such phenomena? These questions, together with others, suggested the desirability of conducting the investigation reported in this paper. Using the spores of 8 fungi a comparative study has been made dealing with the effects of hydrogen- and hydroxyl-ion concentrations upon germination (1) in water, (2) in such single nutrient solutions as mannite and peptone, and (3) in such full nutrient solutions as Czapek's solution and sugar-beet decoction.

---

[1] An investigation carried out at the Missouri Botanical Garden in the Graduate Laboratory of the Henry Shaw School of Botany of Washington University, and submitted as a thesis in partial fulfillment of the requirements for the degree of doctor of philosophy in the Henry Shaw School of Botany of Washington University.

LITERATURE

The literature concerning the germination of fungous spores and their subsequent growth and development, as related to the reaction of the medium, has been historically considered by the writer in an earlier paper (Webb, '19). These articles together with certain others—as reviewed below—are completely cited in the bibliography of this paper.

Buller ('06) obtained a high germination percentage with the spores of *Polyporus squamosus* in a malt-wort extract, the reaction of which was slightly acid. He prepared a solution with tap water, meat extract, 0.5 per cent, peptone, 0.5 per cent, grape sugar, 3 per cent, and gelatin, 10 per cent, adding sodium carbonate until distinctly alkaline. The mycelium grew in this mixture more vigorously and branched more frequently than in the malt-wort extract. In a decoction made from the wood of *Acer pseudoplatanus*, no germination occurred during the first 2 days, but about 1 per cent of the spores had germinated on the third day. However, bacteria had developed abundantly in the culture. Buller says: "The impression given me by this exceptional case was that the metabolism of the bacteria had given rise to some substance which to a slight extent had stimulated the spores to germinate." A change in reaction of the medium may have been a contributing factor, but no mention is made concerning this point.

Falck ('12) showed that the acidity of the medium is a conditioning factor for the growth of several species of *Merulius*. The same writer further observed that *Coniophora*, by making the medium decidedly acid and thus providing favorable conditions for the germination of the spores, conduced to the subsequent growth and development of *Merulius*.

Peltier ('12) obtained best development of *Botrytis cinerea* on various culture media possessing a slightly acid reaction and poorest growth on strongly alkaline media. A strongly alkaline medium, moreover, caused the mycelium to remain sterile, while a strongly acid one reduced the number of sclerotia and favored conidial production.

Stakman ('13), in an extensive study on the germination of cereal smuts, found that the period required for germination and the morphological features during germination varied with the organism. In general, greater vigor of germination and more abundant and prolonged production of sporidia occurred in sugar solutions than in water.

According to Cooley ('14), *Sclerotinia cinerea* may even grow on a medium as acid as the natural juice of sour plums or cherries, although it develops more luxuriantly on a somewhat less acid medium. No perceptible growth was immediately produced with a neutral reaction of the medium, but at the expiration of 2 weeks the mycelial growth at such a reaction nearly equaled that on the acid medium. Spore production was very abundant on the acid media, but entirely inhibited on the alkaline side. While the fungus required relatively high acidity for maximum growth, it could adjust itself in time to a slight degree of alkalinity.

Gillespie ('18) studied the growth of certain strains of *Actinomyces chromogenus* as related to various hydrogen-ion concentrations within the range P_H 4.8–7.2, using succinate, citrate, and potato-tartrate media. In general, the potato-scab organism was found to be inhibited in culture media with an acidity of P_H 5.2, and better growth developed at less acid reactions. Individual strains showed different sensitiveness to acidity, but the differences failed to furnish any consistent distinctions. Growth in the most acid cultures was accompanied by a marked decrease of acidity in the medium, but such changes varied with the organism, the medium, and the initial reaction.

Steinberg ('19), using Pfeffer's solution as a medium, conducted experiments dealing with the relation of 7 different acids to the growth and spore production of *Aspergillus niger*. The addition of acid to the normal nutrient solution, which initially tests P_H 3.0–4.0, produced an acceleration of growth; the addition of alkali to the normal nutrient solution resulted in a diminution in yield. The effect of the increased acidities of the cultures was to cause a retardation or suppression of spore formation as contrasted with the effect of decreased acidity, which failed to exhibit the same phenomenon. All 7 acids gave similar results, indicating that the results are primarily due to the hydrogen ions rather than to the anions.

Zeller, Schmitz, and Duggar ('19), growing various wood-destroying fungi on Czapek's solution, Dunham's solution, a pine decoction, Reed's solution, Richards' solution, and sap from *Acer saccharinum*, whose reactions were adjusted by means of mono-, di-, and tri-basic potassium phosphate, found that there is a decided indication of the advisability of selecting a specific medium for each fungus. The hydrogen-ion concentration did not seem to be the only limiting factor in growth, and the shifting of the hydrogen-ion concentration due to metabolism depended both upon the medium and the fungus. No general statement, in their opinion, could be made concerning the relation between hydrogen-ion concentration of the culture media and the growth of wood-destroying fungi as a group.

Thiel and Weiss ('20) report that acetic acid in aqueous solution exerts a pronounced stimulating effect on the germination of teliospores of *Puccinia graminis*. Other acids and chemical agents in various concentrations were employed by them. but all gave negative results. Although no hydrogen-ion determinations were made, or at least published, the authors are inclined to believe that the process is not one of hydrogen-ion catalysis, but one of a specific activator.

Zeller ('20) obtained a high percentage of spore germination of *Lenzites saepiaria* upon the shavings of short-leaf pine sapwood when the humidity of the air was sufficiently high to supply free water as a film on the wood surface. A decoction prepared by steaming such shavings with distilled water in a reflux tested

P_H 4.2, with active acidity of the shavings probably not above P_H 3.8. This closely approaches P_H 3.0, the optimum hydrogen-ion concentration in various media, as reported in this paper by the writer.

Hopkins ('21), varying the hydrogen-ion concentration of a synthetic medium, the name of which does not appear in the published abstract, by means of KH_2PO_4, K_2HPO_4, H_3PO_4, H_2SO_4, KOH, and NaOH, found that the amount of growth of *Gibberella Saubinetii* increased with decreasing acidity from P_H 2.5 to a maximum at P_H 4.0–4.5. It then decreased to a minimum at P_H 5.0–5.5 and rose again to a second maximum, but the highest point was not determined. Conidial germination of the same organism also showed a double maximum. A *Fusarium* isolated from scabby wheat, but not proved to be *Gibberella*, exhibited a similar depression in the growth-acidity curve. These relations agree well with those established in this paper.

Armstrong ('21), using a modified Richards' solution containing sulphur supplied as $Na_2S_2O_3$ and as $MgSO_4$, determined the growth relations of certain fungi as influenced by hydrogen-ion concentration. Within the experimental range P_H 3.0–7.1, he found that *Aspergillus niger* produced maximum growth with an initial reaction of P_H 5.5–5.9, *Penicillium cyclopium* at P_H 4.1–4.5, and *Botrytis cinerea* at P_H 4.1–5.9. Inhibition of growth, in all cases, occurred at P_H 3.0, and in the case of *Botrytis cinerea*, distinctly retarded and suppressed growth was evidenced at P_H 7.1. Changes in reaction of the liquid culture media invariably took place during growth of the organisms, and, in addition, reversions in the reaction frequently manifested themselves, the nature and degree of such changes and reversions varying with the organism, the medium, and the initial hydrogen-ion concentration.

Karrer ('21), growing certain fungi on Czapek's solution with soluble starch as the source of energy, accumulated interesting data with respect to the reaction of the medium. *Fusarium* produced no growth at P_H 2.0 and the limit on the alkaline side extended beyond P_H 9.2. *Colletotrichum Gossypii* gave a fair quantity of mycelium from between P_H 3.0–4.5 to above P_H 9.2. *Penicillium italicum* displayed a more limited range for favorable growth. Best results were obtained from P_H 2.5 to P_H 4.5, and only a few hyphae were produced at P_H 8.0. During the growth of the organisms, certain shifts in the reaction of the medium were evidenced. In the initially acid cultures, *Fusarium* and *Colletotrichum* produced a shift towards alkalinity, while the reaction of the initially alkaline cultures remained more or less stable; in the case of *Penicillium* the initially acid cultures tended to shift towards neutrality.

MATERIALS AND TECHNIQUE

ORGANISMS

Regarding the fungi and the media employed, the original plan of study was designed primarily to be one of a general nature. Fungi possessing widely divergent cultural relations with respect to the reaction of the medium, that is, fungi producing maximum growth at markedly different hydrogen-ion concentrations, would naturally furnish, it was realized, opportunity for the most interesting data; hence these were given chief consideration. In addition, it seemed desirable to select such economically important forms as might suitably serve to represent certain significant fungous groups. Accordingly, although only relatively few fungi were obtained which would germinate freely in water, the following were chosen and, from previous knowledge of their behavior, differentiated into provisional groups as follows: Acid forms—*Botrytis cinerea*, *Aspergillus niger*, *Penicillium cyclopium*, *P. italicum*, *Puccinia graminis*, and *Lenzites saepiaria*; acid and alkaline forms—*Fusarium* sp.; alkaline forms—*Colletotrichum Gossypii*. The fungi mentioned were obtained from various sources: *B. cinerea*, isolated from lettuce plants growing in the greenhouse; *A niger* and *P. cyclopium* from a jar of beans which had become contaminated in the laboratory; *P. italicum*, from oranges grown in California; *P. graminis*, a strain which was obtained from Berkeley, California; *L. saepiaria*, from sporophores collected at intervals from railroad ties in the vicinity of St. Louis; *Fusarium* sp., isolated from a cotton boll; and *C. Gossypii*, furnished by the South Carolina Agricultural Experiment Station.

In the test-tube cultures from which the spores were taken, *B. cinerea*, *A. niger*, *P. cyclopium*, and *Fusarium* sp. were grown on potato agar made according to Duggar, Severy, and Schmitz ('17); i. e., 230 gms. of potato were cut into small pieces, autoclaved in 1 liter of water for 1 hour at 15 pounds pressure, and filtered while hot. Fifteen gms. of agar were then added, and the mixture was autoclaved for 15 minutes at 15 pounds pressure, and, correction having been made for loss of water, was finally tubed, sterilized, and slanted. *Penicillium italicum* frequently produced spores with difficulty, and the limiting factor seemed to be the reaction of the medium. A medium of relatively high acidity is essential for sporulation with this organism, and the medium here employed was 1.5 per cent agar made up in Czapeks' full nutrient solution as outlined on page 290. *Colletotrichum Gossypii* was grown on agar conforming to a formula suggested by Professor Barre: peptone, 10.0 gms.; glucose, 15.0 gms.; $MgSO_4$, 0.25 gm.; K_2HPO_4, 0.25 gm.; agar, 15.0 gms.; and H_2O, 1000 cc. Cultures grown in the light produced abundant spores; those grown in subdued light, relatively few; and those in the dark, none. All cultures were allowed to grow at room temperature, and the spores were always taken from cultures ranging in age from 10 to 15 days.

In the case of *P. graminis*, wheat seedlings were inoculated with uredospores and allowed to develop within a cheese-cloth cage. Spores to be used for germination were always taken from fresh sori, usually appearing from 10 to 18 days after inoculation. Spores of *L. saepiaria* were obtained according to the pure culture method outlined by Zeller ('16). The sporophores were first rinsed several times in sterile distilled water in large test-tubes and then allowed to stand for about an hour in sterile distilled water. Following this they were removed with sterile forceps, the surplus water being removed by drying with sterile tissue toweling, and were finally placed, hymenium downward, in large, dry, sterile Petri dishes. After 24–48 hours the sporophores had discharged sufficient spores to make a white spore print.

CULTURE SOLUTIONS

Mannite.—The titration curves of the various liquid media employed in this study are shown in fig. 1. The composition of the single nutrient or mannite culture solutions was based primarily upon the Clark and Lubs ('17) titration curve of orthophosphoric acid, and the methods of procedure are the same as those previously described by the writer ('19). Stock solutions of M/5 mannite in M/10 H_3PO_4 and M/5 mannite in N/5 NaOH were made. Equal quantities of the M/5 mannite-M/10 H_3PO_4 solution were placed in each Pyrex flask, and successively increasing proportions of M/5 mannite-N/5 NaOH were added. The flasks were plugged with cotton, sterilized at 15 pounds pressure for 15 minutes, and allowed to stand at least 24 hours before making H-ion determinations. Perfect agreement between calculated and determined values always resulted except within the range P_H 8.0–10.0 where a shift towards neutrality frequently occurred. Such deviations, however, might well be attributed to the extreme lack of buffer action within this range, as shown by fig. 1, and also to any hydrolysis of mannite that might occur during sterilization. A relatively small precipitate invariably occurred in the extreme alkaline cultures.

Czapek's solution.—Czapek's full nutrient solution was made according to the formula published in a recent paper by Zeller, Schmitz, and Duggar ('19): $MgSO_4$ $7H_2O$, 0.5 gm.; KH_2PO_4, 1.0 gm.; KCl, 0.5 gm.; $NaNO_3$, 2.0 gms.; $FeSO_4$, 0.01 gm.; cane sugar, 30.0 gms.; H_2O, 1000 cc. The original nutrient solution was prepared by adding 600 cc., instead of 1000 cc., of distilled water. This method, which has been described by Karrer and Webb ('20), and which will be restated briefly, allows dilutions of the various solutions by the addition of regulated amounts of acid or alkali and water for the adjustment of various H-ion concentrations without materially affecting the concentrations of the nutrient salts or constituents. The acid and alkali used with this particular medium were N/5 HCl and N/20 KOH. This strength of alkali is more satisfactory than one more concentrated, the latter introducing difficulties in securing those reactions less acid than P_H 4.5–5.2—the H-ion concentration

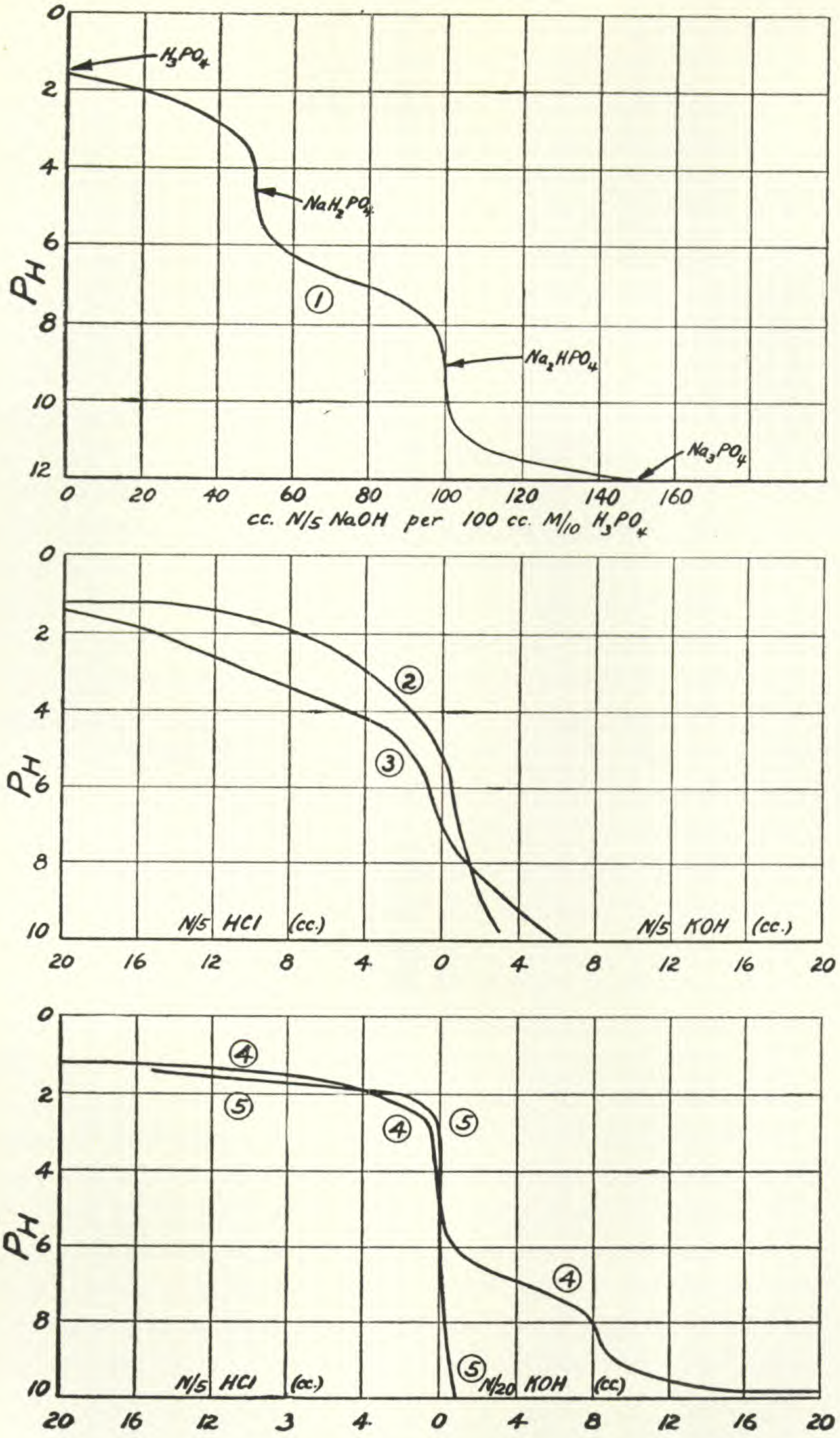

Fig. 1. Titration curves of certain liquid media: (1) orthophosphoric acid (after Clark and Lubs); (2) sugar beet decoction; (3) 2 per cent bacto-peptone solution; (4) Czapek's full nutrient solution (2, 3, and 4 after Karrer and Webb): (5) "water HCl or KOH."

of normal Czapek's solution. With the peptone and the beet decoction solutions, however, N/5 KOH has been used. In all of the media, namely, Czapek, peptone, beet decoction, and water (adjusted by HCl), N/5 HCl was favorable for varying the reactions on the acid side.

Inasmuch as sugars generally react with acid and alkali when heated under pressure, the nutrient solutions and the acid and the alkali were sterilized separately. Thirty cc. of the nutrient solution together with the desired amount of water, the amount of which equals the difference between the total volume and the sum of the volumes of the nutrient solution and the acid or alkali to be added, as shown by fig. 1, were put into small flasks plugged with cotton, and sterilized. After cooling ,the cultures were removed to a culture room, and the definite amounts of sterile acid and alkali were added with sterile graduated pipettes. The final volume of each culture was 50 cc., and each case represented a dilution of the constituents comparable with that in the original nutrient solution. The solutions were allowed to stand at least 24 hours in order to reach a state of equilibrium before H-ion determinations were made. A certain small amount of precipitate occurred upon the addition of alkali, the amount increasing with increase of added alkali. Mannite was substituted for cane sugar, called for in the formula, in order that the same carbohydrate might be present in both the single and the full nutrient solutions, the plan being to make the experiments with the 2 types of media as comparable as possible.

Peptone.—A 2 per cent bacto-peptone solution, prepared according to the titration curve of Karrer and Webb ('20), was used in this investigation. The 2 per cent bacto-peptone solution normally tests P_H 7.0, and the hydrogen-ion concentration was varied with N/5 HCl and N/5 KOH. The buffer action, it will be noticed, is relatively slight on the alkaline side, and is even less apparent in the range P_H 5.0–7.0, the addition of a few tenths of a cubic centimeter of acid producing an almost vertical ascent in the titration curve. With increasing acidity above P_H 5.0, however, buffer action is relatively strong. No precipitation was evidenced in the alkaline cultures of this medium.

Beet Decoction.—The sugar beet decoction was prepared according to the method outlined by Duggar, Severy, and Schmitz ('17). This consists essentially of 370.4 gms. of sugar beets per liter of distilled water, autoclaved at 15 pounds for 1 hour and then filtered. Karrer and Webb's ('20) titration curve and method of varying the hydrogen-ion concentration have been used as a basis, and the detailed remarks for Czapek's full nutrient solution adequately suffice in this case. The beet decoction was made in concentrated form and in sufficient quantity for all of the experiments here reported during the late summer of 1920. This decoction, with proper dilution and without addition of either acid or alkali, tested P_H 5.2, and the H-ion concentration was varied on either side by regulated amounts of N/5 HCl and N/5 KOH. The buffer action on the alkaline side is extremely weak, in fact, much weaker than that of bacto-peptone. Without doubt N/20 alkali would have been better than N/5 for the adjustment of reactions in this medium. The stock solutions of beet decoction, however, were made before this point was determined, and it seemed unwise to change the plan at this stage. No noticeable precipitate immediately occurred in the alkaline cultures, although precipitation did occur upon prolonged standing, and a decided color change from pale yellow to amber was noted as the reaction passed from acid to alkaline.

Water.—Distilled water was adjusted to different hydrogen-ion concentrations by two methods: (1) by successively increasing additions of N/5 NaOH to equal quantities of M/10 H_3PO_4, (2) by additions of acid or alkali to distilled water. The adjustments in the first case were arranged on the principle described for the mannite culture solutions and the term "water H_3PO_4 and NaOH" has been applied to water so regulated. In the other case distilled water was brought to the required P_H value by means of N/5 HCl or N/20 KOH and the term "water HCl or KOH" has been employed to designate these solutions. Distilled water testing P_H 5.2–5.4 was the best that could be obtained. The titration curve, as presented, was worked out very hastily and no special precautions have been taken. Despite the fact that it may be subject to some criticism, it is included for completeness. Initial fluctuations in H-ion concentration generally occurred in the neutral and alkaline cultures.

Mannite and Beet Decoction.—In a few experiments, a medium composed of a mixture of mannite and of beet decoction—the two in different proportions in different series— has been used. The method of preparation is a combination of those used with mannite and with beet decoction. First, a series with mannite and one with beet decoction were made. Then equal volumes of the mannite solutions of varying P_H were placed in flasks and to each was added twice the volume of beet decoction with similar or closely agreeing P_H. In the other series one volume of beet decoction was first added, and then twice the volume of mannite with the corresponding H-ion concentration. The resulting media were accordingly (1) 33⅓ per cent mannite plus 66⅔ per cent beet decoction and (2) 66⅔ per cent mannite plus 33⅓ per cent beet decoction.

Hydrogen-ion concentration determinations of all colorless solutions were made at room temperature according to the usual colorimetric method of Clark and Lubs ('17). The solutions to be tested were, in all cases, allowed to stand at least 24 hours at room temperature in order to establish an equilibrium. Owing to the presence of color in the peptone and in the beet decoction solutions, it was necessary to use a colorimeter for the H-ion determinations. Two types of instruments, a Duboscq (micro) and a Kober have been used at various times in this work, the detailed method of which has been described by Duggar ('19). The H-ion values appearing in the charts, curves, and manuscript, unless otherwise specified, represent initial determinations and not final determinations. Certain changes in reaction during germination occur, but this phase of the topic will be considered subsequently in the discussion.

Solutions ranging in H-ion concentration from P_H 1.2 to P_H 9.2–10.0+ were thus obtained with each medium. Experimental values were, in most cases, identical with the calculated ones, and where the H-ion concentration was beyond the range of the extreme indicator, the reaction has been designated by adding "+" to the last value denoted by the indicator. With any medium the 12 or 13 solutions of varying H-ion concentrations constituted a series. Certain definite P_H values regarded as important for this study, as well as for other physiological studies of the fungi, were desired for each medium, for instance, P_H 3.0, the optimum concentration for germination of certain forms in mannite, as shown by previous studies; several concentrations greater than P_H 3.0, in order to determine inhibiting concentrations; P_H 6.5 for the influence of slight acidity; P_H 7.0 for neutrality; and P_H 7.5 for slight alkalinity. Aside from these desired concentrations the range P_H 1.2–10.0+ has been conveniently but not equally subdivided.

Similar technique to that described in my previous paper (Webb, '19) was used throughout this study. For any organism, and usually for several organisms, a stock solution of a specific medium was made up at one time, so that all data in this paper represent uniformity for an organism and a particular medium.

The initial P_H of any stock solution varied somewhat, variations of several tenths not being infrequent despite the most careful technique during the preparation and the use of highest purity chemicals. Since the highest purity monobasic potassium phosphate then obtainable exhibited a high acidity, the salt was recrystallized until the Sörensen coefficient of P_H 4.529 for 1/15 molecular was obtained.

Doubly distilled water testing P_H 5.2–5.4, distilled first from a Bourdillon still with a block tin condenser, and then redistilled in Pyrex flasks containing several crystals of $KMNO_4$ and condensed in the usual glass condenser, has been used in all of the experiments here reported.

METHOD OF CULTURE

The methods employed, essentially those described by Clark ('99) and Duggar ('01), are substantially those used in my previous work (Webb, '19) and need not be described again. All cultures were run in duplicate and a series with every organism was incubated at three different temperatures, a range of 4–5° C. on either side of an approximate optimum temperature being sufficient for all purposes.

The spores of *C. Gossypii*, it was found, germinate with difficulty in hanging-drop cultures, but germinate more readily in thin films placed on slides. The slides bearing the spore suspension were elevated upon corks in large Petri dishes, and relatively large quantities of the solution to be tested were placed in the bottom of the dishes. The cultures being destroyed upon examination, it was possible to make only one reading.

In testing the change in reaction of the medium during germination, 2-cc. portions of the various solutions of a series were placed under aseptic conditions in sterile test-tubes. The tubes had a volume of 22 cc. and were plugged with cotton. Several loops of spores were placed in each test-tube of solution, care being exercised to secure a heavy and uniform suspension, and, following inoculation, the cultures were allowed to incubate at a provisional optimum temperature for a period of 20 hours. All of the cultures were set up in duplicate and controls were run simultaneously. The initial and final hydrogen-ion concentrations were determined in the usual way.

Extreme precautions were exercised in the care and the cleaning of glassware. A detailed description of such operations may be found in the writer's earlier paper (Webb, '19).

EXPERIMENTAL DATA

Cultures were examined at different intervals, depending on the length of time required for the spores of the particular fungus to germinate, as determined from preliminary experiments. Spore counts were made from 5 different fields of the hanging

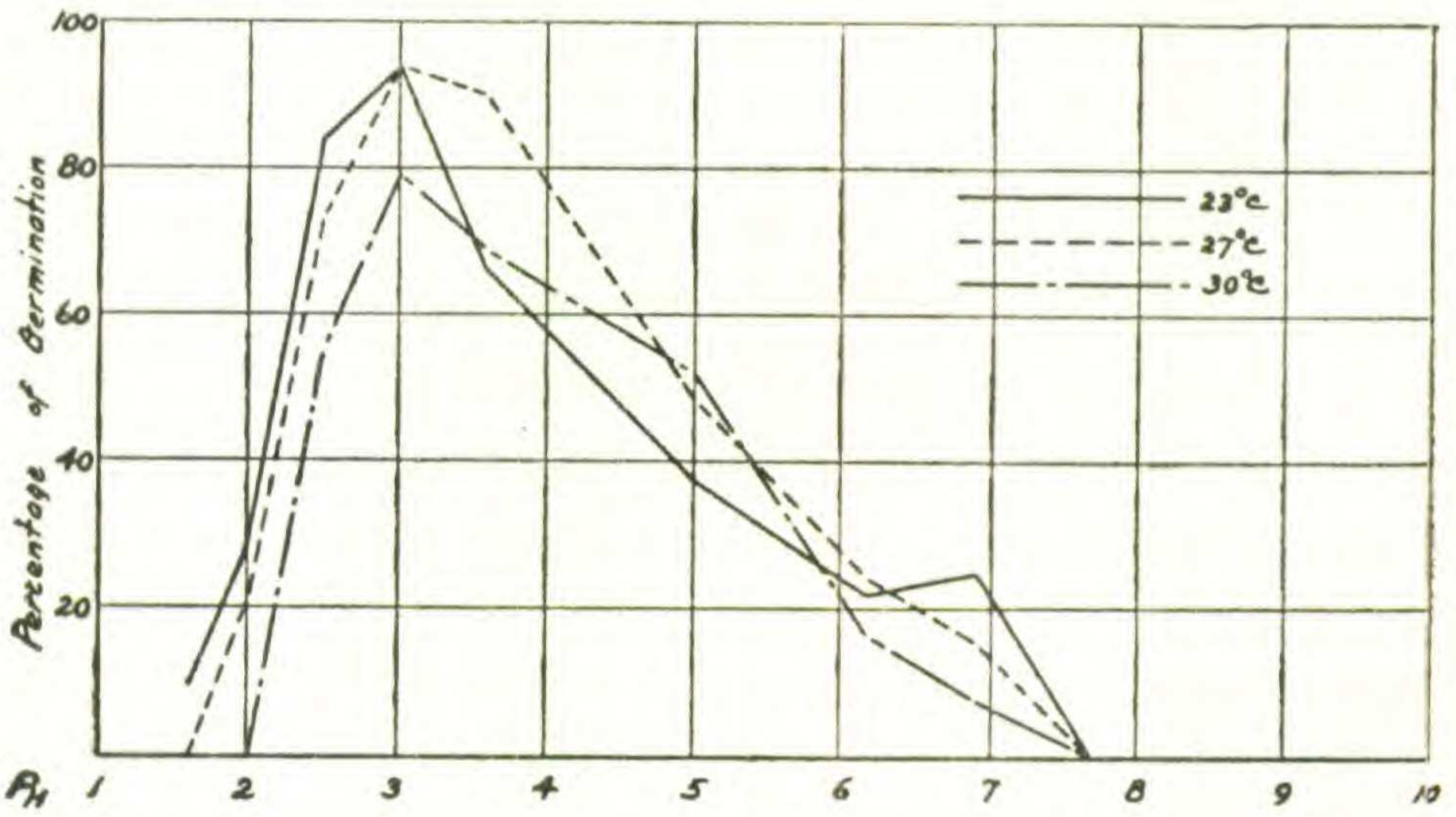

Fig. 2. *Botrytis cinerea* in M/5 mannite solution.

drop, involving usually from 50 to 100 spores, and the average percentage of germination recorded. Readings were made with each set of cultures at two different incubation periods, and a third one would have been made had not luxuriant mycelial growth in the nutrient solutions prevented. Values obtained from the second reading are considered as reliable as those that might have been obtained from a third reading; in fact, data which were accumulated in the preliminary study with incubation periods as denoted by second and third readings showed close agreement, indicating, therefore, that germination was sufficiently and satisfactorily completed at this period. As might be ex-

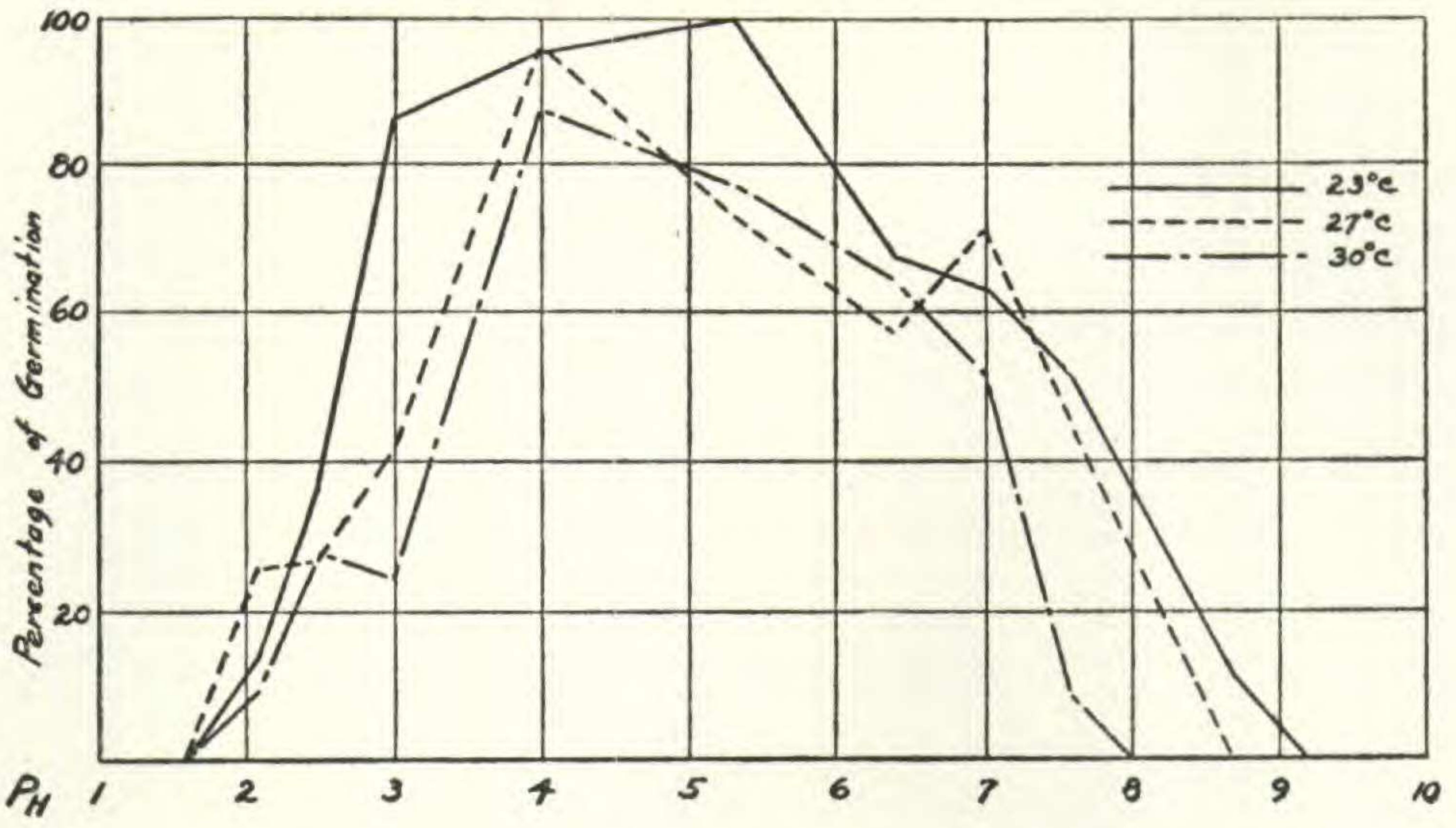

Fig. 3. *Botrytis cinerea* in 2 per cent bacto-peptone solution.

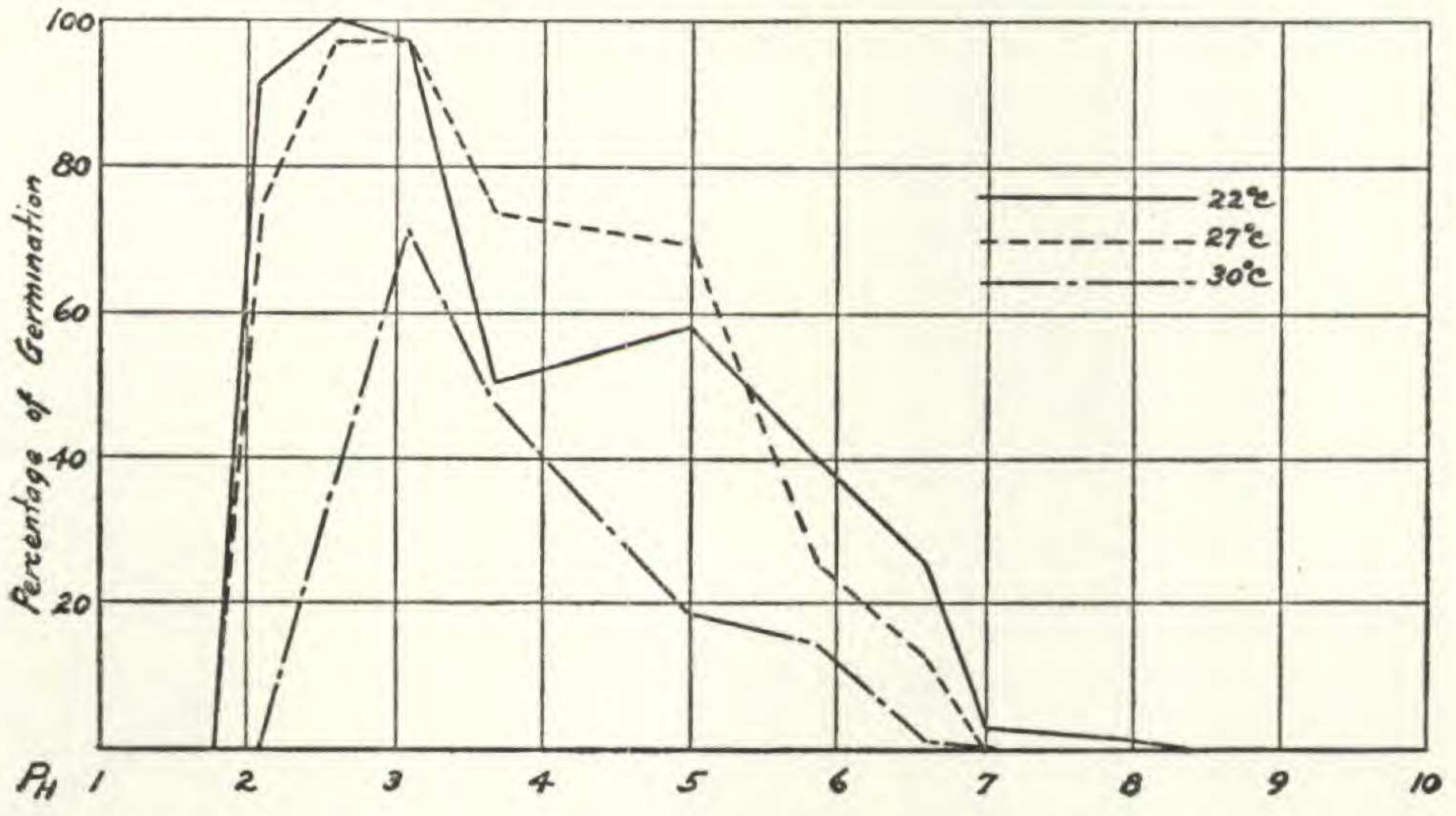

Fig. 4. *Botrytis cinerea* in Czapek's full nutrient solution.

pected, fluctuations sometimes apparently erratic and seemingly unexplainable occurred occasionally. In such cases, the experiments were always duplicated and frequently triplicated.

The curves are developed from the percentage averages, as indicated, each curve representing the final reading of the germination quantities of a particular organism at a definite temperature in a certain medium. The percentages of spore germination are plotted as ordinates and the hydrogen-ion concentration of the solutions as abscissae. Curves are shown giving germination percentages at each temperature and with each medium. In addition an assembled graph is presented, this being made by averaging the germination percentages of the three temperatures with each of the media.

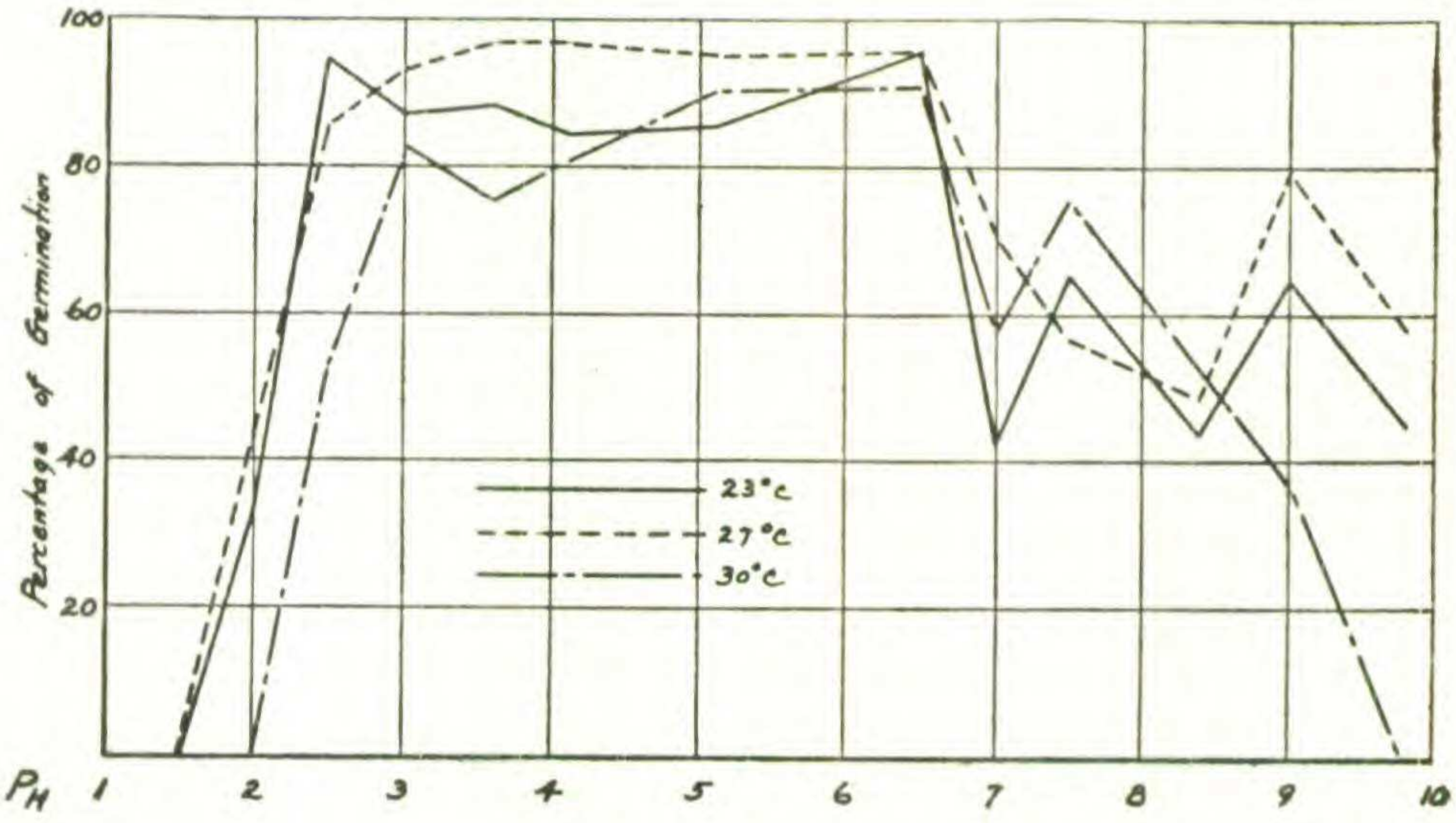

Fig. 5. *Botrytis cinerea* in sugar beet decoction.

In examining the experimental results, it must be borne in mind that perfect germination is not to be expected with these fungi in water or in solutions containing mannite and the acid or the alkali as the only nutrients. Dextrose, in fact, would have yielded higher germination percentages than did mannite, but it is not certain that it would remain stable with the treatment given.

Of the various liquid media employed in this study, the sugar beet decoction has undoubtedly furnished the most interesting data. Germination obtained with the acid cultures has not been unusual, but that obtained with the alkaline cultures has been most striking.

The spores of *B. cinerea* germinate in the mannite medium within the range P_H 1.6–6.9 with best germination afforded at P_H 3.0 and similar data are obtained in Czapek's full nutrient solution. The relations in the peptone solution are similar, except that there is a slight shift of the germination-acidity curve towards alkalinity. Decidedly different data are furnished with the sugar beet decoction, maximum germination extending in a zone between P_H 3.0 and P_H 6.5 and ranging on the alkaline side beyond P_H 9.6. Germination in "water HCl or KOH" agrees with that in the sugar beet decoction (though not stimulated to such a great extent) in that germination freely occurs under conditions of active alkalinity. This is in direct contrast with the other media employed where no or relatively feeble germination is obtained under such conditions. The relations in "water H_3PO_4 and NaOH" are very similar to those in the mannite solution, but the composition of the two media, it must be remembered, is the same except for the presence and absence of mannite.

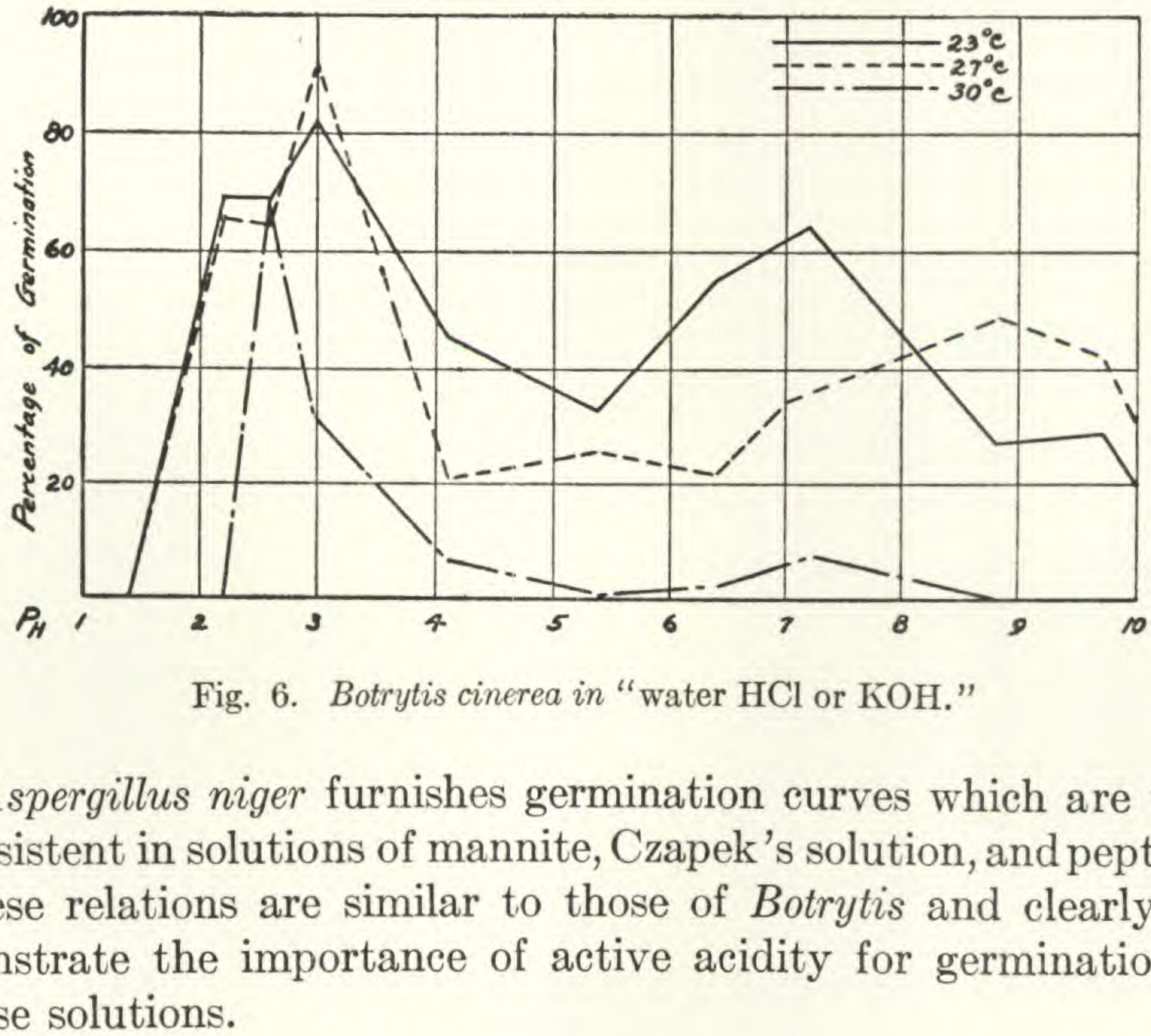

Fig. 6. *Botrytis cinerea in* "water HCl or KOH."

Aspergillus niger furnishes germination curves which are very consistent in solutions of mannite, Czapek's solution, and peptone. These relations are similar to those of *Botrytis* and clearly demonstrate the importance of active acidity for germination in these solutions.

The spores of *Lenzites saepiaria* germinate freely in acid media, and this is the only case where the sugar beet decoction fails to stimulate germination in the alkaline cultures.

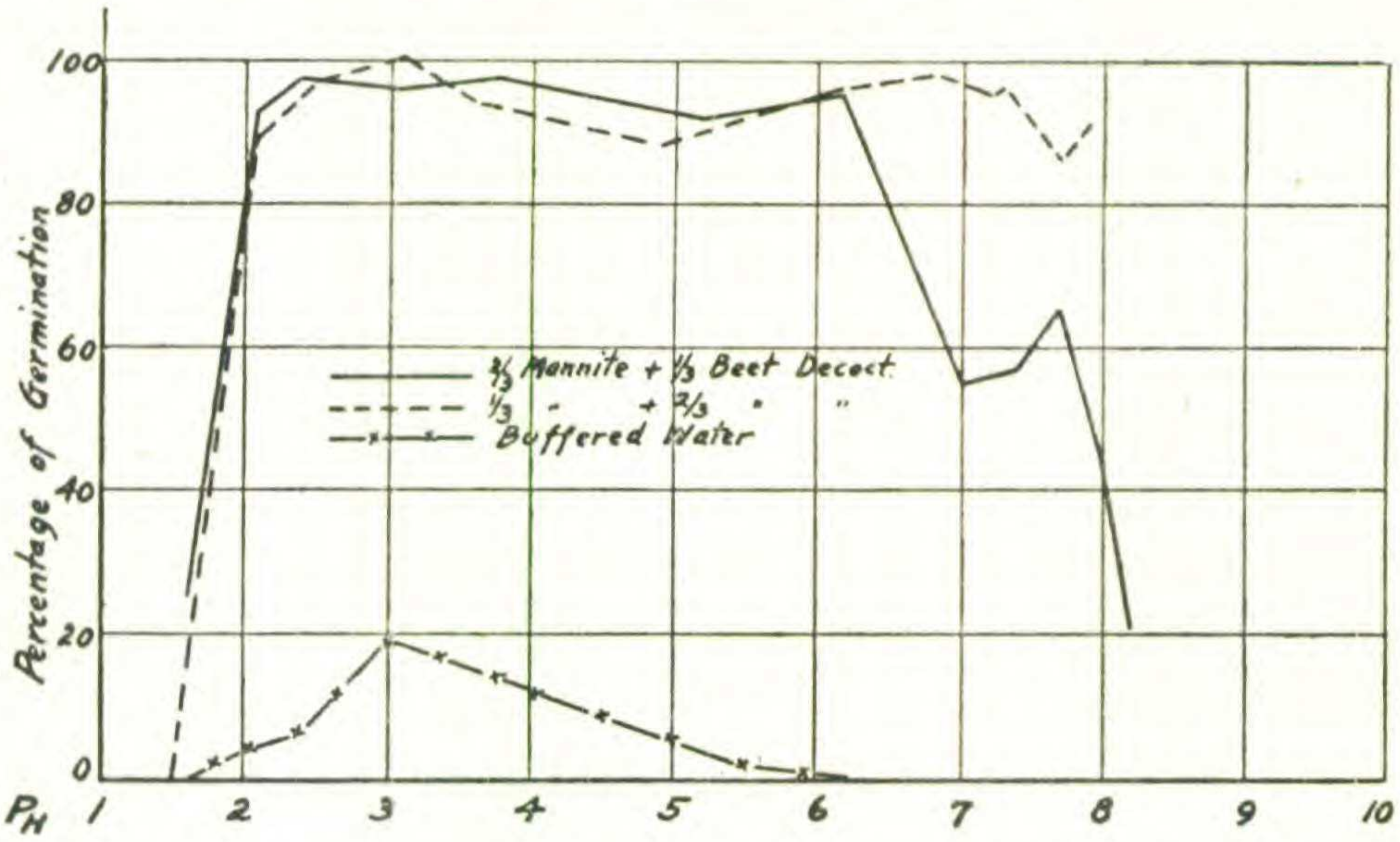

Fig. 7. *Botrytis cinerea* in (1) a medium composed of M/5 mannite solution and sugar beet decoction and (2) "water H_3PO_4 and NaOH" (25° C.).

Penicillium cyclopium does not appear to be as dependent upon the stimulating effects of hydrogen ions as *Aspergillus* and *Botrytis*, and the same to a greater degree may be said of

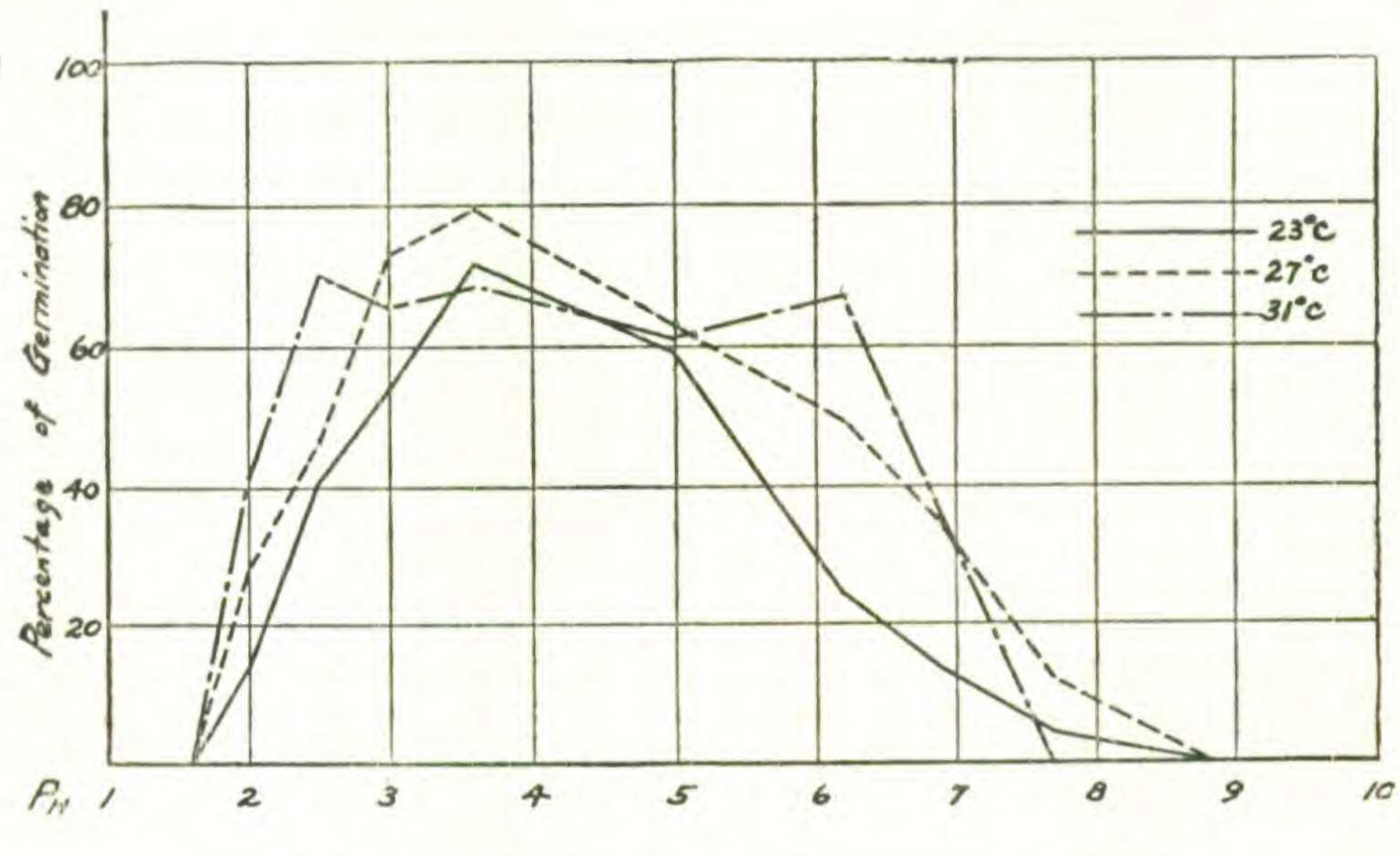

Fig. 8. *Aspergillus niger* in M/5 mannite solution.

Fusarium sp. The 2 organisms referred to, *Penicillium* and *Fusarium*, are conspicuous for their double maxima; one on the acid side, and the other near neutrality.

Relatively high active acidity in the synthetic culture media favors spore germination of *Penicillium italicum*, but slightly alkaline conditions in the sugar beet decoction are equally as favorable, if not more so, than acid conditions in the same medium.

In general, a slightly acid or neutral reaction affords most perfect germination of the uredospores of *Puccinia graminis*.

Germination of the spores of *Colletotrichum Gossypii* is very variable and no definite curve is produced, but an alkaline reaction of the medium is most favorable.

The effects of hydrogen-ion concentration upon the germination of these fungous spores in the different culture media are shown in tables I–IX, and such relations are graphically presented in figs. 2–39. Tables I to IX follow.

TABLE I

BOTRYTIS CINEREA. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

Temp.	Hrs.	Hydrogen-ion concentration, P_H													Media
		1.6	2.0	2.5	3.0	3.6	5.0	6.2	6.9	7.7	8.8	9.6	10.0+		
23° C.	6	0.0	0.0	29.4	63.8	41.9	26.9	14.4	1.9	0.0	0.0	0.0	0.0		Mannite
	20	9.5	28.0	83.5	93.1	66.0	37.5	21.9	24.5	0.0	0.0	0.0	0.0		
27° C.	6	0.0	0.0	38.3	65.1	75.2	33.0	10.3	1.5	0.0	0.0	0.0	0.0		
	20	0.0	21.0	72.3	93.6	89.5	48.1	24.0	15.6	0.0	0.0	0.0	0.0		
30° C.	6	0.0	0.0	0.0	17.0	6.0	4.2	1.0	0.0	0.0	0.0	0.0	0.0		
	20	0.0	0.0	54.9	79.1	68.4	52.1	16.3	7.6	0.0	0.0	0.0	0.0		
		1.2	1.8	2.1	2.6	3.1	3.7	5.0	5.9	6.6	7.0	7.9	8.4	9.4	Czapek
22° C.	6	0.0	0.0	88.0	93.5	91.0	44.5	66.0	14.5	22.0	0.0	0.0	0.0	0.0	
	20	0.0	0.0	91.1	100.0	96.5	50.4	58.3	39.5	25.8	2.6	1.2	0.0	0.0	
27° C.	6	0.0	0.0	6.5	89.0	90.0	70.5	58.0	5.0	4.0	0.0	0.0	0.0	0.0	
	20	0.0	0.0	74.0	96.7	96.8	73.7	69.3	25.1	12.2	0.0	0.0	0.0	0.0	
30° C.	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	20	0.0	0.0	0.0	36.3	71.3	47.7	18.4	14.5	1.0	0.0	0.0	0.0	0.0	

TABLE I (cont.)

Temp.	Hrs.	Hydrogen-ion concentration P_H													Media
		1.6	2.1	2.5	3.0	4.0	5.3	6.4	7.0	7.6	8.7	9.2	10.0+		Peptone
23° C.	6	0.0	25.0	39.5	74.4	65.0	69.5	44.0	36.0	4.1	0.0	0.0	0.0		
	20	0.0	23.2	36.2	86.3	95.5	100.0	67.6	63.3	51.3	11.3	0.0	0.0		
27° C.	6	0.0	26.8	26.3	48.0	63.0	55.5	36.2	49.2	10.0	0.0	0.0	0.0		
	20	0.0	25.4	26.1	41.7	96.0	73.6	57.0	71.2	44.3	0.0	0.0	0.0		
30° C.	6	0.0	0.0	29.0	26.0	70.0	58.9	50.3	36.2	5.1	0.0	0.0	0.0		
	20	0.0	9.2	27.5	24.4	87.2	77.5	64.1	51.0	8.4	0.0	0.0	0.0		
		1.5	2.0	2.5	3.0	3.6	4.1	5.1	6.5	7.0	7.5	8.4	9.0	9.8	Beet decoction
23° C.	6	0.0	0.0	46.0	61.3	73.0	70.8	73.4	76.2	28.1	63.3	41.3	2.0	1.0	
	20	0.0	32.7	94.0	86.6	88.0	84.0	85.0	94.9	42.2	65.1	43.1	64.2	44.5	
27° C.	6	0.0	2.8	83.3	85.1	84.4	89.8	98.0	87.4	37.1	54.0	46.9	17.0	0.5	
	20	0.0	43.1	85.5	92.9	96.9	96.9	95.0	95.3	70.0	56.7	48.2	79.2	58.0	
30° C.	6	0.0	0.0	0.0	0.0	0.0	16.8	0.0	33.4	13.7	14.2	0.0	0.0	0.0	
	20	0.0	0.0	52.8	82.4	75.4	80.7	90.0	90.7	58.0	75.7	52.5	36.7	0.0	
		1.4	2.2	2.6	3.0	4.1	5.4	6.4	7.2	8.8	9.7	10+			Water (HCl or KOH)
23° C.	6	0.0	59.1	68.8	74.8	19.8	23.0	53.2	31.0	8.0	7.2	5.0			
	20	0.0	69.5	69.5	82.0	45.6	32.5	55.8	64.9	26.9	28.6	20.0			
27° C.	6	0.0	63.0	69.6	92.4	6.2	24.1	8.4	23.2	36.5	31.0	10.0			
	20	0.0	65.9	64.3	92.0	20.6	25.7	21.9	34.0	49.0	42.5	31.0			
30° C.	6	0.0	0.0	0.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0			
	20	0.0	0.0	68.2	31.3	6.1	0.5	2.0	7.0	0.0	0.0	0.0			
		1.6	2.0	2.4	3.0	4.0	5.5	6.2	7.0	7.7	8.1	8.2	8.6		Water (H_3PO_4 and NaOH)
24° C.	6	0.0	0.0	3.3	18.4	9.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	20	0.0	3.5	6.1	18.7	12.5	1.5	0.0	0.0	0.0	0.0	0.0	0.0		
		1.6	2.1	2.4	3.1	3.8	5.2	6.2	7.0	7.4	7.7	8.0	8.2		⅔ mannite + ⅓ B. decoction
24° C.	6	0.0	45.0	89.1	93.4	90.8	86.6	91.6	18.8	8.2	6.2	1.2	0.0		
	20	25.1	92.0	97.8	95.9	97.5	91.7	95.0	55.3	57.1	65.0	43.8	20.7		
		1.5	2.1	2.5	3.1	3.6	4.9	6.2	6.8	7.2	7.3	7.7	7.9		⅓ mannite + ⅔ B. decoction
24° C.	6	0.0	8.5	42.0	38.0	50.0	57.6	56.4	55.7	22.5	6.0	12.0	1.0		
	20	0.0	88.4	96.4	100.0	94.0	88.0	96.0	98.0	94.7	96.0	86.0	90.7		

TABLE II

ASPERGILLUS NIGER. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS.

Temp.	Hrs.	Hydrogen-ion concentration, P_H													Media
		1.6	2.0	2.5	3.0	3.6	5.0	6.2	6.9	7.7	8.8	9.6	10.0+		Mannite
23° C.	10	0.0	0.0	30.9	43.5	14.5	8.6	4.5	1.2	0.0	0.0	0.0	0.0		
	20	0.0	13.2	40.7	53.9	71.7	58.9	24.0	13.6	4.0	0.0	0.0	0.0		
27° C.	10	0.0	24.8	41.3	65.0	55.9	61.0	41.2	29.9	2.9	0.0	0.0	0.0		
	20	0.0	27.5	46.0	72.9	79.6	62.6	48.8	34.5	12.0	0.0	0.0	0.0		
31° C.	10	0.0	22.8	56.0	55.9	67.0	52.2	67.5	24.5	0.0	0.0	0.0	0.0		
	20	0.0	41.0	70.0	65.9	68.6	61.0	67.5	36.8	0.0	0.0	0.0	0.0		
		1.2	1.8	2.1	2.6	3.1	3.7	5.0	5.9	6.6	7.0	7.9	8.4	9.4	Czapek
22° C.	16	0.0	0.0	5.5	34.5	72.0	84.0	38.5	60.0	38.5	36.5	15.0	5.5	0.0	
	26	0.0	0.0	12.3	29.9	71.7	89.2	41.2	67.1	45.1	42.5	27.4	6.2	0.0	
27° C.	16	0.0	0.0	23.0	32.0	72.0	96.5	39.5	41.0	22.0	25.0	10.5	0.0	0.0	
	26	0.0	0.0	27.7	50.1	88.0	96.0	39.0	68.0	30.0	49.7	12.0	2.0	0.0	
31° C.	16	0.0	0.0	22.5	24.5	74.0	85.0	51.0	65.0	51.0	42.0	7.5	0.0	0.0	
	26	0.0	0.0	33.9	50.1	88.8	81.9	48.3	71.7	53.2	59.9	14.8	0.0	0.0	
		1.6	2.1	2.5	3.0	4.0	5.3	6.4	7.0	7.6	8.7	9.2	10.0+		Peptone
22° C.	10	0.0	0.0	0.0	0.0	32.9	8.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	20	0.0	26.3	26.6	44.8	78.7	32.8	4.8	5.6	0.5	0.0	0.0	0.0	0.0	
27° C.	10	0.0	0.0	0.0	5.7	62.6	35.9	34.4	3.4	0.0	0.0	0.0	0.0		
	20	0.0	29.1	31.0	71.0	66.1	37.3	44.2	6.6	4.4	1.0	0.0	0.0		
31° C.	10	0.0	41.4	86.0	97.3	87.4	58.0	56.3	66.0	24.0	0.0	0.0	0.0		
	20	6.6	89.5	97.5	100.0	93.1	66.8	54.7	65.5	27.0	4.0	0.0	0.0		
		1.5	2.0	2.5	3.0	3.6	4.1	5.1	6.5	7.0	7.5	8.4	9.0	9.8	Beet decoction
23° C.	10	0.0	0.0	4.8	0.5	4.9	10.0	10.0	15.7	25.3	12.8	1.0	0.0	0.0	
	20	66.2	90.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	85.9	70.5	30.8	
27° C.	10	0.0	82.5	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	82.0	43.1	
	20	20.0	95.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	69.0	
31° C.	10	19.3	15.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	98.6	0.0	
	20	45.0	62.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	45.2	
		1.6	2.1	2.4	3.1	3.8	5.2	6.2	7.0	7.4	7.7	8.0	8.2		⅔ mannite + ⅓ B. decoction
24° C.	10	6.2	59.6	87.5	88.5	83.4	85.3	88.8	88.8	65.9	57.4	60.0	19.3		
	20	91.7	92.9	94.0	98.6	97.3	98.4	98.0	98.0	98.0	96.5	96.0	72.6		
		1.5	2.1	2.5	3.1	3.6	4.9	6.2	6.8	7.2	7.7	7.9			⅓ mannite + ⅔ B. decoction
24° C.	10	0.0	6.0	16.0	31.0	30.4	29.3	25.6	15.0	28.4		10.0	6.7		
	20	96.0	100.0	100.0	96.7	100.0	92.4	100.0	100.0	96.0		98.0	97.2		

TABLE III

LENZITES SAEPIARIA. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

Temp.	Hrs.	Hydrogen-ion concentration, P_H													Media
		1.6	2.0	2.5	3.0	3.6	5.0	6.2	6.9	7.7	8.8	9.6	10.0+		
23° C.	18	0.0	0.0	6.0	43.3	57.9	42.2	32.7	11.7	0.0	0.0	0.0	0.0		Mannite
	30	0.0	0.0	36.8	71.8	57.1	53.3	44.1	13.8	0.0	0.0	0.0	0.0		
27° C.	18	0.0	0.0	10.9	48.1	58.4	49.3	34.1	12.2	0.0	0.0	0.0	0.0		
	30	0.0	0.0	51.7	72.1	66.3	55.0	36.8	16.8	0.0	0.0	0.0	0.0		
32° C.	18	0.0	0.0	36.7	62.5	31.1	21.5	24.8	10.9	0.0	0.0	0.0	0.0		
	30	0.0	0.0	49.0	60.0	38.6	31.0	32.0	17.2	0.0	0.0	0.0	0.0		
		1.2	1.8	2.1	2.6	3.2	3.7	4.8	6.0	6.6	7.2	8.0	8.2	9.0	
20° C.	18	0.0	0.0	0.0	6.4	41.9	41.7	30.2	14.4	8.3	0.0	0.0	0.0	0.0	Czapek
	30	0.0	0.0	0.0	28.0	52.5	44.7	32.9	23.0	15.2	0.0	0.0	0.0	0.0	
25° C.	18	0.0	0.0	0.0	1.0	38.2	21.9	22.6	6.9	5.7	0.0	0.0	0.0	0.0	
	30	0.0	0.0	0.0	1.0	53.9	22.3	22.2	11.1	6.6	0.0	0.0	0.0	0.0	
30° C.	18	0.0	0.0	0.0	10.5	34.9	33.9	10.0	8.2	0.0	0.0	0.0	0.0	0.0	
	30	0.0	0.0	0.0	22.1	40.6	31.6	13.4	11.4	0.0	0.0	0.0	0.0	0.0	
		1.6	2.1	2.5	3.0	4.0	5.5	6.6	7.2	7.6	8.7	9.1	10.0+		
23° C.	18	0.0	0.0	51.7	52.4	46.7	26.7	10.0	1.9	0.0	0.0	0.0	0.0		Peptone
	30	0.0	8.9	90.8	91.0	59.2	36.3	13.8	2.0	0.0	0.0	0.0	0.0		
27° C.	18	0.0	0.0	58.8	70.7	70.2	23.9	20.9	4.0	0.0	0.0	0.0	0.0		
	30	0.0	13.4	84.9	94.4	82.0	42.3	31.7	6.0	0.0	0.0	0.0	0.0		
32° C.	18	0.0	0.0	27.5	40.5	32.3	26.9	18.6	3.0	0.0	0.0	0.0	0.0		
	30	0.0	51.8	82.9	92.3	70.0	29.0	25.0	3.8	0.0	0.0	0.0	0.0		
		1.5	2.0	2.5	3.0	3.6	4.1	5.1	6.5	7.0	7.5	8.4	9.0	9.8	
23° C.	18	0.0	28.6	95.0	88.0	73.8	85.0	40.0	41.2	85.6	70.0	0.0	0.0	0.0	Beet decoction
27° C.	18	0.0	76.6	56.6	78.9	84.5	79.8	39.5	35.6	8.9	76.0	0.0	0.0	0.0	
31° C.	18	0.0	17.7	47.5	93.8	62.7	51.0	35.7	43.7	70.9	37.5	0.0	0.0	0.0	

TABLE IV

PENICILLIUM CYCLOPIUM. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

Temp.	Hrs.	Hydrogen-ion concentration, P_H													Media
		1.6	2.0	2.5	3.0	3.6	5.0	6.2	6.9	7.7	8.8	9.6	10.0+		
23° C.	10	0.0	0.0	0.0	0.0	41.9	43.0	53.5	38.2	11.9	0.0	0.0	0.0		Mannite
	20	0.0	4.9	3.0	22.8	62.6	45.8	62.5	54.0	13.4	0.0	0.0	0.0		
27° C.	10	0.0	0.0	0.0	0.0	58.3	37.1	62.4	23.7	13.0	0.0	0.0	0.0		
	20	0.0	45.5	66.2	48.0	52.2	43.8	62.4	54.7	19.5	0.0	0.0	0.0		
31° C.	10	0.0	0.0	0.0	0.0	5.5	8.0	35.1	10.9	0.0	0.0	0.0	0.0		
	20	0.0	0.0	2.0	23.6	25.5	21.1	48.1	35.0	1.0	0.0	0.0	0.0		
		1.2	1.8	2.1	2.6	3.1	3.7	5.0	5.9	6.6	7.0	7.9	8.4	9.4	Czapek
22° C.	18	0.0	0.0	0.0	48.0	85.0	85.5	93.0	80.5	91.5	91.0	74.5	30.0	18.0	
	27	0.0	0.0	0.0	68.6	100.0	97.2	86.3	77.3	94.9	97.6	90.5	45.1	22.1	
27° C.	18	0.0	0.0	3.5	23.5	77.0	73.0	78.5	73.5	70.0	63.5	40.5	12.0	0.0	
	27	0.0	0.0	46.9	67.0	100.0	88.3	90.5	88.3	100.0	63.5	49.0	24.1	0.0	
31° C.	18	0.0	0.0	0.0	0.0	22.0	68.0	37.5	15.5	10.0	16.0	15.0	0.0	0.0	
	27	0.0	0.0	0.0	32.9	66.8	93.8	49.1	28.4	22.2	25.9	18.7	4.2	0.0	
		1.6	2.1	2.5	3.0	4.0	5.3	6.4	7.0	7.6	8.7	9.2	10.0+		Peptone
23° C.	10	0.0	0.0	0.0	2.0	32.2	45.3	46.9	58.7	45.6	9.1	4.1	0.0		
	20	0.0	18.4	22.4	46.1	68.2	74.1	76.7	86.1	88.7	59.1	41.7	12.8		
27° C	10	0.0	0.0	0.0	3.7	40.0	68.4	55.1	53.2	50.9	11.7	2.1	0.0		
	20	0.0	10.6	30.7	54.8	72.5	85.0	85.8	84.8	76.8	49.0	60.2	5.0		
31° C.	10	0.0	0.0	0.0	0.0	5.5	17.9	23.8	1.6	4.0	0.5	0.0	0.0		
	20	0.0	0.0	8.4	8.0	65.6	56.1	49.0	37.6	56.1	36.9	26.4	0.0		
		1.5	2.0	2.5	3.0	3.6	4.1	5.1	6.5	7.0	7.5	8.4	9.0	9.8	Beet decoction
23° C.	10	0.0	0.0	0.0	0.0	1.0	60.0	50.7	52.5	42.8	35.4	17.7	20.0	23.9	
	20	0.0	0.0	45.3	78.8	92.0	100.0	98.0	99.5	100.0	98.0	100.0	100.0	100.0	
27° C.	10	0.0	0.0	0.0	2.0	50.6	81.9	89.1	46.7	23.9	27.0	62.4	43.2	38.1	
	20	0.0	0.0	39.6	79.2	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
31° C.	10	0.0	0.0	0.0	37.5	55.2	73.5	48.4	70.6	38.9	49.0	73.8	76.3	24.0	
	20	0.0	0.0	0.0	55.0	86.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	61.3	

TABLE V

PENICILLIUM ITALICUM. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

Temp.	Hrs.	Hydrogen-ion concentration, P_H													Media
		1.6	2.0	2.5	3.0	3.6	5.0	6.2	6.9	7.7	8.8	9.6	10.0+		Mannite
15° C.	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		
	35	0.0	0.0	0.5	5.4	4.2	0.0	0.0	0.0	3.5	0.0	0.0	0.0		
23° C.	20	0.0	3.3	1.1	5.2	3.7	1.0	0.0	1.0	0.0	0.0	0.0	0.0		
	35	0.0	12.3	4.0	7.7	6.0	2.0	1.0	1.8	15.7	0.0	0.0	0.0		
27° C.	20	0.0	0.0	4.5	6.0	5.7	0.0	0.0	5.0	0.0	0.0	0.0	0.0		
	35	0.0	1.0	6.1	8.0	12.9	3.5	2.0	7.5	20.0	0.0	0.0	0.0		
		1.2	1.8	2.1	2.6	3.2	3.7	4.8	6.0	6.6	7.2	8.0	8.2	9.0	Czapek
15° C.	20	0.0	0.0	0.0	4.0	2.5	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	
	35	0.0	0.0	20.0	38.0	47.6	38.2	4.6	26.7	18.7	2.5	0.5	0.3	0.0	
21° C.	20	0.0	7.5	21.5	44.5	25.3	19.5	2.0	3.9	5.5	2.2	0.0	0.0	0.0	
	35	0.0	55.4	58.2	45.5	42.7	45.3	33.2	39.9	23.7	4.5	3.5	1.5	0.0	
26° C.	20	0.0	0.0	0.0	32.4	5.0	9.2	2.5	9.4	11.5	1.3	0.0	0.0	0.0	
	35	0.0	22.4	22.9	56.9	33.3	30.5	25.4	40.9	17.7	2.8	3.0	0.0	0.0	
		1.6	2.1	2.5	3.0	4.0	5.5	6.6	7.2	7.6	8.7	9.1	10.0+		Peptone
15° C.	10	0.0	0.0	15.3	42.4	55.2	9.5	4.3	0.0	0.0	0.0	0.0	0.0		
	20	22.9	20.9	31.0	76.6	90.9	35.4	24.8	0.0	0.0	0.0	0.0	0.0		
23° C.	10	0.0	0.0	6.4	8.1	34.8	11.6	8.8	2.6	0.0	0.0	0.0	0.0		
	20	14.0	22.7	89.2	89.6	91.6	46.0	40.5	13.0	0.0	0.0	0.0	0.0		
27° C.	10	0.0	0.0	15.0	21.9	65.8	21.0	13.7	4.5	0.0	0.0	0.0	0.0		
	20	3.7	3.8	79.4	85.5	82.9	58.6	46.1	16.6	0.0	0.0	0.0	0.0		
		1.8	2.4	2.7	3.1	3.6	4.2	5.0	5.8	6.0	6.8	8.0	8.6	8.9	Beet decoction
15° C.	20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	35	0.0	47.5	43.5	49.9	49.4	41.7	57.1	41.0	57.4	71.6	74.1	63.8	10.3	
20° C.	20	0.0	13.5	14.5	8.9	31.8	37.8	49.9	42.8	52.8	45.1	10.9	2.2	0.0	
	35	0.0	51.3	59.5	47.4	50.2	61.4	67.0	51.9	48.4	71.5	65.4	52.8	59.1	
25° C.	20	0.0	5.6	8.0	36.8	65.0	47.1	46.0	42.0	60.7	60.0	54.0	28.0	0.0	
	35	0.0	49.6	30.4	48.3	64.6	58.4	54.4	44.3	57.3	63.8	60.6	60.0	15.0	

TABLE VI

PUCCINIA GRAMINIS. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

Temp.	Hrs.	Hydrogen-ion concentration, P_H													Media
		1.6	2.0	2.5	3.0	3.6	5.0	6.2	6.9	7.7	8.8	9.6	10.0+		Mannite
12° C.	6	0.0	0.0	0.0	2.0	42.6	39.1	40.0	9.1	0.0	0.0	0.0	0.0		
	20	0.0	0.0	0.0	2.0	58.0	52.4	59.8	15.5	0.0	0.0	0.0	0.0		
15° C.	6	0.0	0.0	0.0	0.0	30.2	70.0	55.6	48.1	1.7	0.0	0.0	0.0		
	20	0.0	0.0	2.0	16.1	41.6	75.4	54.2	55.7	2.0	0.0	0.0	0.0		
23° C.	6	0.0	0.0	0.0	24.9	51.1	65.4	35.0	42.5	2.5	0.0	0.0	0.0		
	20	0.0	0.0	0.5	50.8	57.7	67.4	39.9	51.9	6.7	0.0	0.0	0.0		
		1.2	1.8	2.1	2.5	3.0	3.9	5.0	5.9	6.6	7.1	8.0	8.5	9.2	Czapek
12° C.	6	0.0	0.0	0.0	0.0	42.0	96.5	65.0	87.5	63.5	34.0	14.0	0.0	0.0	
	18	0.0	0.0	0.0	0.0	59.4	94.0	65.0	95.0	61.0	46.0	45.0	0.0	0.0	
16° C.	6	0.0	0.0	0.0	0.0	72.5	92.0	65.0	89.5	64.5	40.5	14.5	3.5	0.0	
	18	0.0	0.0	0.0	0.0	79.4	97.8	68.7	89.6	77.3	51.9	45.0	4.7	0.0	
22° C.	6	0.0	0.0	0.0	0.0	0.0	57.0	44.5	71.0	57.5	41.5	13.5	0.0	0.0	
	18	0.0	0.0	0.0	0.0	0.0	72.4	50.0	73.9	56.6	46.6	24.4	3.3	0.0	
		1.6	2.1	2.5	3.0	4.0	5.5	6.6	7.2	7.6	8.7	9.1	10.0+		Peptone
12° C.	6	0.0	0.0	0.0	0.0	68.4	85.2	87.4	76.5	43.2	0.0	0.0	0.0		
	20	0.0	0.0	0.0	28.4	78.2	90.3	96.7	81.7	56.3	0.0	0.0	0.0		
15°C.	6	0.0	0.0	0.0	0.0	29.2	70.5	55.6	45.0	31.2	0.0	0.0	0.0		
	20	0.0	0.0	0.0	0.0	65.7	89.5	78.0	58.5	62.0	0.0	0.0	0.0		
23° C.	6	0.0	0.0	0.0	0.0	21.4	70.2	48.8	59.1	37.9	0.0	0.0	0.0		
	20	0.0	0.0	0.0	0.0	54.6	72.5	50.0	58.6	50.5	0.0	0.0	0.0		
		1.5	2.0	2.5	3.0	3.6	4.1	5.1	6.5	7.0	7.5	8.4	9.0	9.8	Beet decoction
12° C.	6	0.0	0.0	0.0	0.0	36.7	71.6	50.0	46.2	63.5	69.0	63.7	0.0	0.0	
	20	0.0	0.0	0.0	10.7	37.9	77.5	55.0	45.5	79.2	74.0	75.0	13.9	0.0	
15° C.	6	0.0	0.0	0.0	0.0	15.9	83.8	83.7	59.2	69.7	62.5	20.0	0.0	0.0	
	20	0.0	0.0	0.0	0.0	18.8	82.9	85.1	65.9	81.7	65.7	50.9	25.2	0.0	
23° C.	6	0.0	0.0	0.0	0.0	40.8	52.0	60.6	38.9	62.2	56.7	53.4	0.0	0.0	
	20	0.0	0.0	0.0	0.0	38.9	52.0	66.1	42.5	71.2	72.6	52.4	0.0	0.0	
		1.2	2.1	2.5	3.0	3.9	5.2	5.8	6.8	8.0	9.4				Water (HCl or KOH)
10° C.	6	0.0	0.0	3.8	39.2	43.0	37.1	43.0	33.1	38.7	25.5				
	18	0.0	0.0	19.6	73.4	70.6	65.2	62.5	46.0	40.8	28.0				
16° C.	6	0.0	0.0	0.0	0.0	70.3	66.6	50.2	56.9	56.7	31.4				
	18	0.0	0.0	0.0	8.4	72.1	73.2	53.2	68.7	57.9	30.8				
22° C.	6	0.0	0.0	19.2	48.2	55.0	49.1	41.9	34.5	39.6	25.0				
	18	0.0	0.0	22.6	60.0	57.0	57.6	53.2	45.0	43.0	22.5				

TABLE VII

FUSARIUM SP. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

Temp.	Hrs.	Hydrogen-ion concentration, P_H													Media
		1.6	2.0	2.5	3.0	3.6	5.0	6.2	6.9	7.7	8.8	9.6	10.0+		Mannite
23° C.	6	0.0	0.0	0.0	0.0	24.3	58.8	65.4	69.3	12.2	12.2	17.0	2.0		
	20	0.0	0.0	2.5	34.0	58.8	64.2	62.0	71.3	31.9	34.1	49.0	39.0		
27° C.	6	0.0	0.0	0.0	1.2	29.2	33.5	47.2	51.3	8.3	0.0	1.0	0.0		
	20	0.0	0.0	4.4	43.2	59.8	58.8	64.8	81.0	31.2	26.3	40.9	22.7		
32° C.	6	0.0	0.0	0.0	0.0	13.0	27.2	30.9	28.2	0.0	0.0	0.0	0.0		
	20	0.0	0.0	7.5	21.6	41.4	48.7	68.8	70.0	11.3	8.6	5.2	4.9		
		1.2	1.8	2.1	2.6	3.1	3.7	5.0	5.9	6.6	7.0	7.9	8.4	9.4	Czapek
22° C.	6	0.0	0.0	0.0	0.0	79.5	69.0	67.5	82.0	83.0	54.0	72.0	91.0	73.0	
	20	0.0	0.0	0.0	83.2	100.0	89.7	83.4	81.5	86.2	66.3	89.4	97.8	82.4	
27° C.	6	0.0	0.0	0.0	0.0	58.0	86.0	83.5	83.5	86.5	78.5	93.0	92.0	86.0	
	20	0.0	0.0	0.0	82.6	91.9	91.9	84.3	92.4	93.7	97.2	100.0	100.0	84.3	
31° C.	6	0.0	0.0	0.0	0.0	81.5	72.0	65.5	67.0	83.0	61.0	82.0	89.5	76.5	
	20	0.0	0.0	0.0	68.8	100.0	83.9	80.8	80.0	97.8	79.5	86.7	100.0	84.3	
		1.6	2.1	2.5	3.0	4.0	5.3	6.4	7.0	7.6	8.7	9.2	10.0+		Peptone
23° C.	6	0.0	0.0	0.0	0.0	60.2	90.5	77.5	75.8	92.8	77.4	76.9	71.9		
	20	0.0	21.2	55.0	65.5	100.0	100.0	100.0	100.0	100.0	87.5	90.0	90.0		
27° C.	6	0.0	0.0	0.0	0.0	66.3	82.7	87.2	61.7	83.7	89.3	89.5	65.2		
	20	0.0	16.0	46.0	71.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0		
31° C.	6	0.0	0.0	0.0	0.0	44.0	69.0	50.7	67.7	64.6	62.6	64.0	63.8		
	20	0.0	8.9	10.0	55.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0		
		1.5	2.0	2.5	3.0	3.6	4.1	5.1	6.5	7.0	7.5	8.4	9.0	9.8	Beet decoction
23° C.	6	0.0	0.0	0.0	0.0	5.0	15.0	34.2	39.6	63.8	54.6	49.9	43.3	48.7	
	20	0.0	0.0	11.0	37.1	76.7	85.0	100.0	100.0	100.0	100.0	92.5	92.5	92.5	
27° C.	6	0.0	0.0	0.0	0.0	16.0	28.5	52.1	50.0	62.6	86.7	68.0	55.0	66.0	
	20	0.0	0.0	0.0	8.8	78.6	90.0	100.0	100.0	100.0	100.0	95.0	95.0	95.0	
31° C.	6	0.0	0.0	0.0	0.0	0.0	10.0	25.0	24.0	27.0	48.7	31.3	32.9	27.5	
	20	0.0	0.0	0.0	0.0	5.0	52.3	77.2	78.8	95.0	100.0	86.3	81.0	76.0	

TABLE VII (cont.)

Temp.	Hrs.	Hydrogen-ion concentration, P_H													Media
		1.4	2.2	2.6	3.0	4.1	5.4	6.4	7.2	8.8	9.7	10.0+			Water (HCl or KOH)
23° C.	6	0.0	0.0	0.0	69.1	36.3	29.4	62.5	51.7	55.0	47.7	36.5			
	20	0.0	30.3	24.8	83.5	51.4	47.4	74.0	60.0	60.0	48.6	53.7			
27° C.	6	0.0	0.0	51.3	52.8	43.9	43.7	55.1	64.4	40.7	43.9	47.5			
	20	0.0	0.0	81.1	85.2	70.7	56.5	53.3	65.0	50.8	55.2	71.3			
31° C.	6	0.0	0.0	5.0	21.6	4.7	8.2	26.5	35.4	15.3	8.9	7.0			
	20	0.0	13.0	12.5	30.0	14.0	11.9	59.9	47.4	22.8	19.6	30.3			
		1.6	2.0	2.4	3.0	4.0	5.5	6.2	7.0	7.7	8.1	8.2	8.6		(Water H_3PO_4 and NaOH)
24° C.	6	0.0	0.0	0.0	2.0	37.6	38.5	27.2	24.1	27.4	22.3	10.0	8.2		
	20	0.0	13.7	20.0	27.1	76.4	76.6	58.3	68.5	73.2	41.2	40.0	15.0		

TABLE VIII

COLLETOTRICHUM GOSSYPII. AVERAGE PERCENTAGES OF SPORE GERMINATION IN CERTAIN LIQUID CULTURE MEDIA AT DIFFERENT TEMPERATURES AND AT VARIOUS HYDROGEN-ION CONCENTRATIONS

Temp.	Hrs.	Hydrogen-ion concentration, P_H													Media
		1.2	1.8	2.1	2.6	3.2	3.7	4.8	6.0	6.6	7.2	8.0	8.2	9.0	Czapek
23° C.	10	0.0	0.0	0.0	0.0	18.5	6.4	11.7	23.6	15.7	16.9	9.3	11.0	5.0	
27° C.	10	0.0	0.0	0.0	0.0	5.2	11.1	12.1	12.6	14.3	17.5	10.4	12.7	4.0	
31° C.	10	0.0	0.0	0.0	0.0	6.7	16.6	21.2	17.6	18.7	18.3	18.0	29.0	18.9	
		1.6	2.1	2.5	3.0	4.0	5.5	6.6	7.2	7.6	8.7	9.1	10.0+		Peptone
23° C.	10	0.0	0.0	0.0	0.0	0.0	28.3	14.6	21.9	24.7	25.3	0.0	0.0		
27° C.	10	0.0	0.0	0.0	0.0	0.0	29.4	21.2	21.8	28.4	24.4	18.0	13.6		
31° C.	10	0.0	0.0	0.0	0.0	0.0	40.2	27.4	29.8	27.5	30.0	22.3	14.1		
		1.5	2.0	2.5	3.0	3.6	4.1	5.1	6.5	7.0	7.5	8.4	9.0	9.8	Beet decoction
23° C.	10	0.0	0.0	0.0	0.0	0.0	0.5	8.9	25.3	25.0	21.9	27.3	25.7	22.6	
27° C.	10	0.0	0.0	0.0	0.0	0.0	3.0	11.7	39.3	25.0	22.3	19.5	24.1	35.2	
31° C.	10	0.0	0.0	0.0	0.0	0.0	8.5	14.3	26.9	27.6	27.4	32.6	26.1	49.3	

TABLE IX

ASSEMBLED DATA

Organisms	Percentage of germination		Hydrogen-ion concentration, P_H			Media
	Primary maximum	Secondary maximum	Primary optimum	Secondary optimum	Range of germination	
Aspergillus niger	73.3		3.6		2.0– 7.7	Mannite
	89.0	68.7	3.7	5.9	2.1– 8.4	Czapek
	79.3		4.0		*1.6– 8.7	Peptone
	100.0		2.5–7.5		*1.5– 9.8*	Beet decoction
					1.6– 8.2	⅔ mannite + ⅓ beet decoction
					1.5– 7.9	⅓mannite+⅔beet decoction
Penicillium cyclopium	57.7	46.8	6.2	3.6	2.0– 7.7	Mannite
	93.1	72.4	3.7	6.6	2.1– 9.4*	Czapek
	73.9	70.0	7.6	4.0–7.0	2.1–10.0+*	Peptone
	100.0		4.1–9.0		2.5–9.8*	Beet decoction
Botrytis cinerea	88.8		3.0		*1.6– 6.9	Mannite
	88.2		3.1		2.1– 7.9	Czapek
	92.9		4.0		2.1– 8.7	Peptone
	89.0		3.0–6.5		2.0– 9.8*	Beet decoction
	68.0	35.3	2.5–3.0	7.2	2.1–10.0+*	Water (HCl or KOH)
	18.7		3.0		2.0– 5.5	Water(H_3PO_4 and NaOH)
	95.0		2.4–6.2		*1.6– 8.2*	⅔mannite+⅓beet decoction
	100.0	96.2	3.1	6.2–7.3	2.1– 7.9*	⅓mannite+⅔beet decoction

TABLE IX (cont.)

Organisms	Percentage of germination		Hydrogen-ion concentration, P_H			Media
	Primary maximum	Secondary maximum	Primary optimum	Secondary optimum	Range of germinatin	
Fusarium sp.	74.1		6.9		2.5–10.0+*	Mannite
	99.3	97.3	8.4	3.1	2.6– 9.4*	Czapek
	98.7		4.0-10.0+		2.1–10.0+*	Peptone
	99.1		7.0–7.5		2.5– 9.8*	Beet decoction
	66.2	62.4	3.0	6.4	2.1–10.0+*	Water(HCl or KOH)
	76.5	73.2	4.0–5.5	7.7	2.0– 8.6*	Water (H_3PO_4 and NaOH)
Penicillium italicum	13.1	7.3	7.7	3.0–3.6	2.0– 7.7	Mannite
	46.8	35.8	2.6	6.0	1.8– 8.2	Czapek
	86.2		3.0–4.0		*1.6– 7.0	Peptone
	67.8	56.0	6.8–8.0	3.6–5.0	2.4– 9.8*	Beet decoction
Lenzites saepiaria	68.0		3.0		2.5– 6.9	Mannite
	49.0		3.2		2.7– 6.6	Czapek
	92.6		3.0		2.1– 7.2	Peptone
	87.6	61.2	3.0	7.5	2.0– 7.5	Beet decoction
Puccinia graminis	65.1		5.0		2.5– 7.7	Mannite
	88.1	86.2	3.7	5.9	3.1– 8.4	Czapek
	84.1		5.5		3.0– 7.6	Peptone
	69.8	77.4	4.1–5.1	7.0	3.0– 9.0	Beet decoction
	65.4		3.9–5.8		2.5– 9.4*	Water (HCl or KOH)
Colletotrichum Gossypii					3.0– 3.6	Mannite
					3.2– 9.0*	Czapek
					5.5–10.0+*	Peptone
					4.1– 9.8*	Beet decoction

* Germination obtained in the most acid or in the most alkaline cultures of the experiment.

The relatively high percentages and the extended range of germination as related to hydrogen-ion concentration in a beet decoction medium are very striking, and the extended range of germination in "water HCl or KOH" is equally striking. It would seem, therefore, that either the beet decoction might possess some stimulating substance, which is absent in the synthetic culture media, or else it might undergo changes in reaction during the process of spore germination. Germination

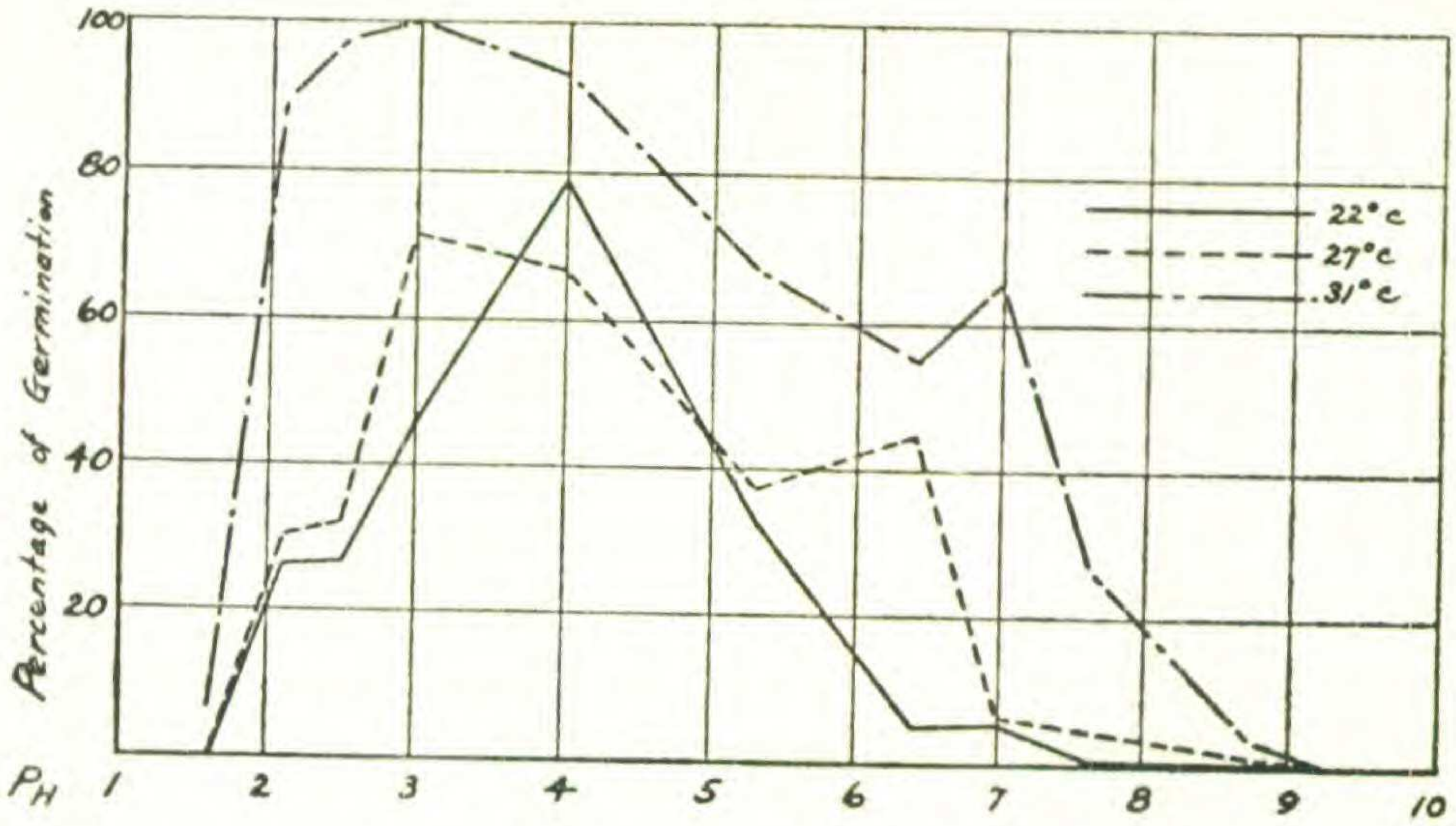

Fig. 9. *Aspergillus niger* in 2 per cent bacto-peptone solution.

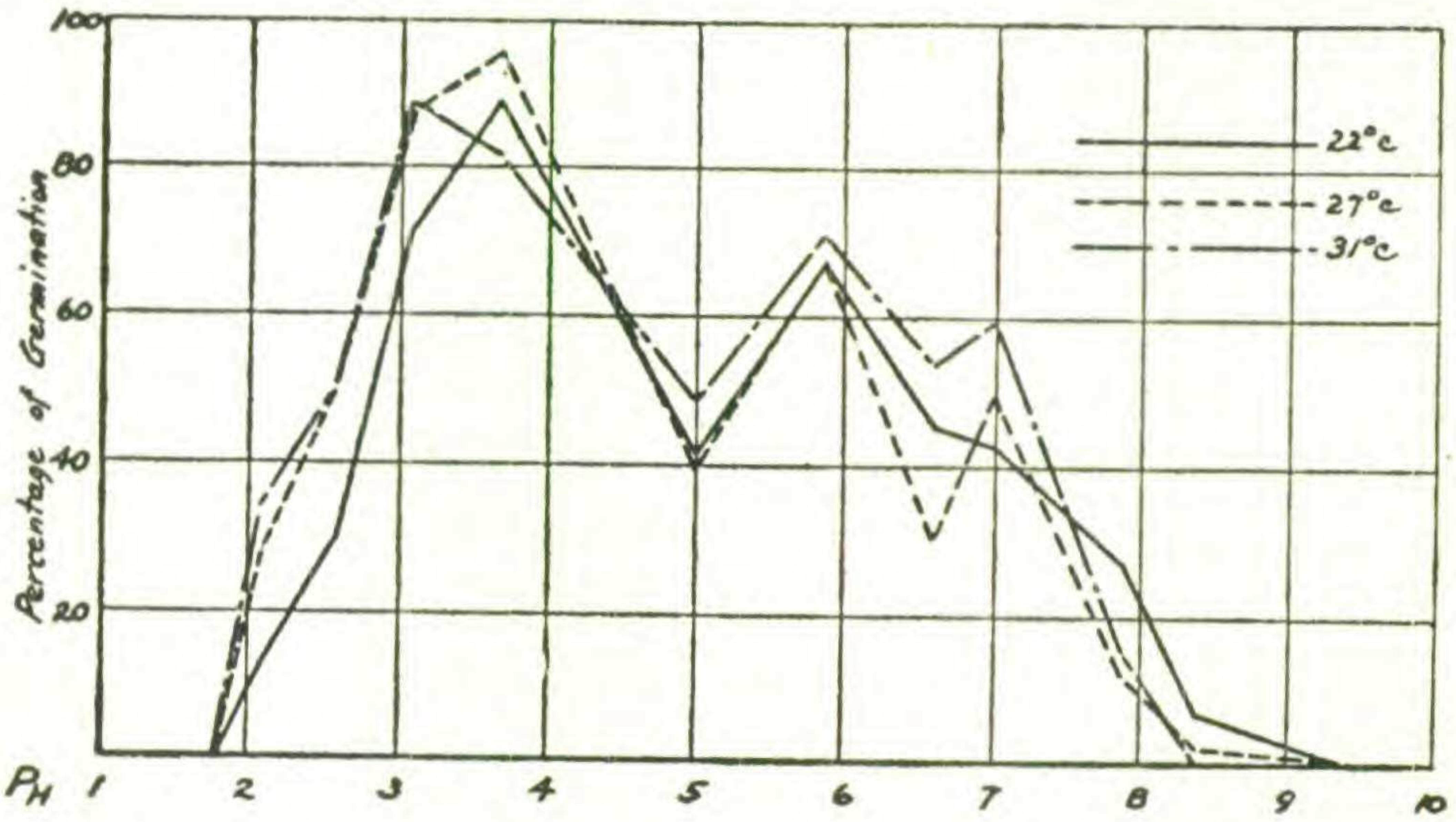

Fig. 10. *Aspergillus niger* in Czapek's full nutrient solution.

percentages are relatively low in "water HCl or KOH", but the expansion in range beyond that offered by the other culture solutions—with the exception, of course, of beet decoction—would also tend to indicate a change in reaction during germination. In view of these facts, it seemed advisable to conduct a few simple experiments which might throw light on this problem.

Inasmuch as *A. niger* and *B. cinerea* furnished such widely different results in solutions of mannite and beet decoction, these two organisms were selected for a study of the first aspect mentioned, and the extremely stimulating effect of beet decoction upon germination of these spores is shown by the data accumulated from experiments involving mixtures of the mannite and beet decoction solutions in various proportions, as presented in the tables for the respective organisms.

The same organisms, together with *Fusarium* sp., were employed in studying the second possibility, that is, changes in the reaction of the medium during germination. These data appear in tables x, xi, and xii, and will be considered subsequently in the discussion.

TABLE X

BOTRYTIS CINEREA. INITIAL AND FINAL HYDROGEN-ION CONCENTRATIONS DURING GERMINATION FOR 20 HOURS AT 24° C.

Hydrogen-ion concentration, P_H														Media
Initial	1.6	2.0	2.5	3.0	4.0	5.3	6.2	7.0	7.5	8.1	8.6	9.6		Mannite
Final	1.6	2.0	2.5	3.0	4.0	5.3	6.2	7.0	7.5	8.0	8.2	9.1		
Control	1.6	2.0	2.5	3.0	4.0	5.3	6.2	7.0	7.5	8.1	8.2	9.0		
Initial	1.2	1.8	2.1	2.8	3.4	3.6	5.0	6.1	6.7	7.2	8.0	8.9	9.6	Czapek
Final	1.2	1.8	2.1	2.8	3.4	3.6	5.0	6.1	6.7	7.2	7.8	8.2	9.1	
Control	1.2	1.8	2.1	2.8	3.4	3.6	5.0	6.1	6.7	7.2	7.8	8.2	9.0	
Initial	1.8	2.4	2.7	3.1	3.6	4.2	5.0	5.8	6.5	6.8	8.0	8.6	8.9	Beet decoction
Final	1.8	2.3	3.6	3.8	3.8	4.7	4.8	5.5	5.7	6.4	6.7	7.9	8.6	
Control	1.8	2.4	2.7	3.2	3.8	4.4	5.0	5.8	6.6	7.0	8.1	8.3	8.8	
Initial	1.6	2.0	2.4	3.0	4.0	5.5	6.2	7.0	7.7	8.1	8.2	8.6		Water (H_3PO_4 &NaOH)
Final	1.6	2.0	2.4	3.0	4.1	5.5	6.2	7.0	7.6	8.1	8.2	8.6		
Control	1.6	2.0	2.4	3.0	4.1	5.5	6.2	7.0	7.7	8.1	8.2	8.6		
Initial	1.4	2.0	2.4	2.9	4.0	5.1	5.5	6.3	6.6	6.9	6.9	7.1	8.6	Water (HCl or KOH)
Final	1.4	2.0	2.4	3.2	6.0	6.7	6.8	6.7	6.9	7.1	7.1	7.1	7.6	
Control	1.4	2.0	2.4	2.9	4.6	6.2	6.6	6.7	6.9	7.1	7.2	7.3	7.7	

TABLE XI

ASPERGILLUS NIGER. INITIAL AND FINAL HYDROGEN-ION CONCENTRATIONS DURING GERMINATION FOR 20 HOURS AT 24° C.

	Hydrogen-ion concentration, P_H													Media
Initial	1.6	2.0	2.5	3.0	4.0	5.3	6.2	7.0	7.5	8.1	8.6	9.6		Mannite
Final	1.6	2.1	2.6	3.1	4.0	5.4	6.3	7.0	7.1	8.1	8.2	9.0		
Control	1.6	2.0	2.5	3.0	4.0	5.3	6.2	7.0	7.5	8.1	8.2	9.0		
Initial	1.2	1.8	2.1	2.8	3.4	3.6	5.0	6.1	6.7	7.2	8.0	8.9	9.6	Czapek
Final	1.2	1.8	2.1	2.8	3.5	3.6	5.2	6.2	6.8	7.2	7.8	8.1	8.9	
Control	1.2	1.8	2.1	2.8	3.4	3.6	5.0	6.1	6.7	7.2	7.8	8.2	9.0	
Initial	1.8	2.4	2.7	3.1	3.6	4.2	5.0	5.8	6.5	6.8	8.0	8.6	8.9	Beet decoction
Final	1.8	2.4	2.8	3.7	3.9	4.5	4.9	5.5	5.7	5.9	6.4	6.4	7.4	
Control	1.8	2.4	2.7	3.2	3.8	4.4	5.0	5.8	6.6	7.0	8.1	8.3	8.8	

TABLE XII

FUSARIUM SP. INITIAL AND FINAL HYDROGEN-ION CONCENTRATIONS DURING GERMINATION FOR 20 HOURS AT 27° C.

	Hydrogen-ion concentration, P_H													Media
Initial	1.6	2.0	2.4	3.0	4.0	5.3	6.2	7.0	7.6	8.0	8.2	8.8	9.6	Mannite
Final	1.6	2.0	2.4	3.0	4.0	5.3	6.2	7.0	7.5	7.9	8.1	8.5	9.0	
Control	1.6	2.0	2.4	3.0	4.0	5.3	6.2	7.0	7.6	8.0	8.2	8.5	9.0	
Initial	1.2	1.8	2.1	2.8	3.4	3.6	5.0	6.1	6.7	7.2	8.0	8.9	9.6	Czapek
Final	1.2	1.8	2.1	2.8	3.5	4.2	5.6	6.2	6.7	7.2	7.6	8.1	8.8	
Control	1.2	1.8	2.1	2.8	3.4	3.6	5.0	6.1	6.7	7.2	7.8	8.2	9.0	
Initial	1.5	2.0	2.4	2.9	4.0	5.5	6.2	7.0	7.7	8.1	8.3	8.6		Water (H_3PO_4 &NaOH)
Final	1.5	2.0	2.4	2.9	(4.4)	(5.6)	(6.3)	7.0	7.7	8.2	8.3	8.7		
Control	1.5	2.0	2.4	3.0	4.6	5.5	6.2	7.0	7.7	8.1	8.3	8.7		
Initial	1.4	2.0	2.4	2.9	4.0	5.1	5.5	6.3	6.6	6.9	6.9	7.1	8.6	Water (HCl or KOH)
Final	1.4	2.1	2.5	5.5	6.7	6.9	7.2	7.1	7.2	7.2	7.3	7.5	7.7	
Control	1.4	2.0	2.4	2.9	4.6	6.2	6.6	6.7	6.9	7.1	7.2	7.3	7.7	

DISCUSSION

Germination of fungous spores is a subject that has engaged the attention of botanists—physiologists and pathologists—for many years. Various aspects of the problem have been extensively studied, and certain factors, such as temperature, light, moisture, and reaction of the medium have been experimentally considered. The toxic properties of H and OH ions have been treated more or less, but hydrogen-ion concentration has so recently become biologically important that the influence of this factor in germination was practically new. Having no technique they could apply for the direct determination of hydrogen-ion concentration, the earlier investigators, like many of the later ones, frequently employed conductivity data in making their interpretations. This method is, however, inapplicable when other solutes are introduced, and the presence of strong buffers, whether inorganic or organic, would render most difficult any computation of active acidity or alkalinity.

It is believed that the results here presented are sufficient to change materially the prevailing view as to the relation of spore germination to acid and alkaline media. Although germination, as related to active acidity and alkalinity, varies with the organism and with the liquid culture medium, it is a process

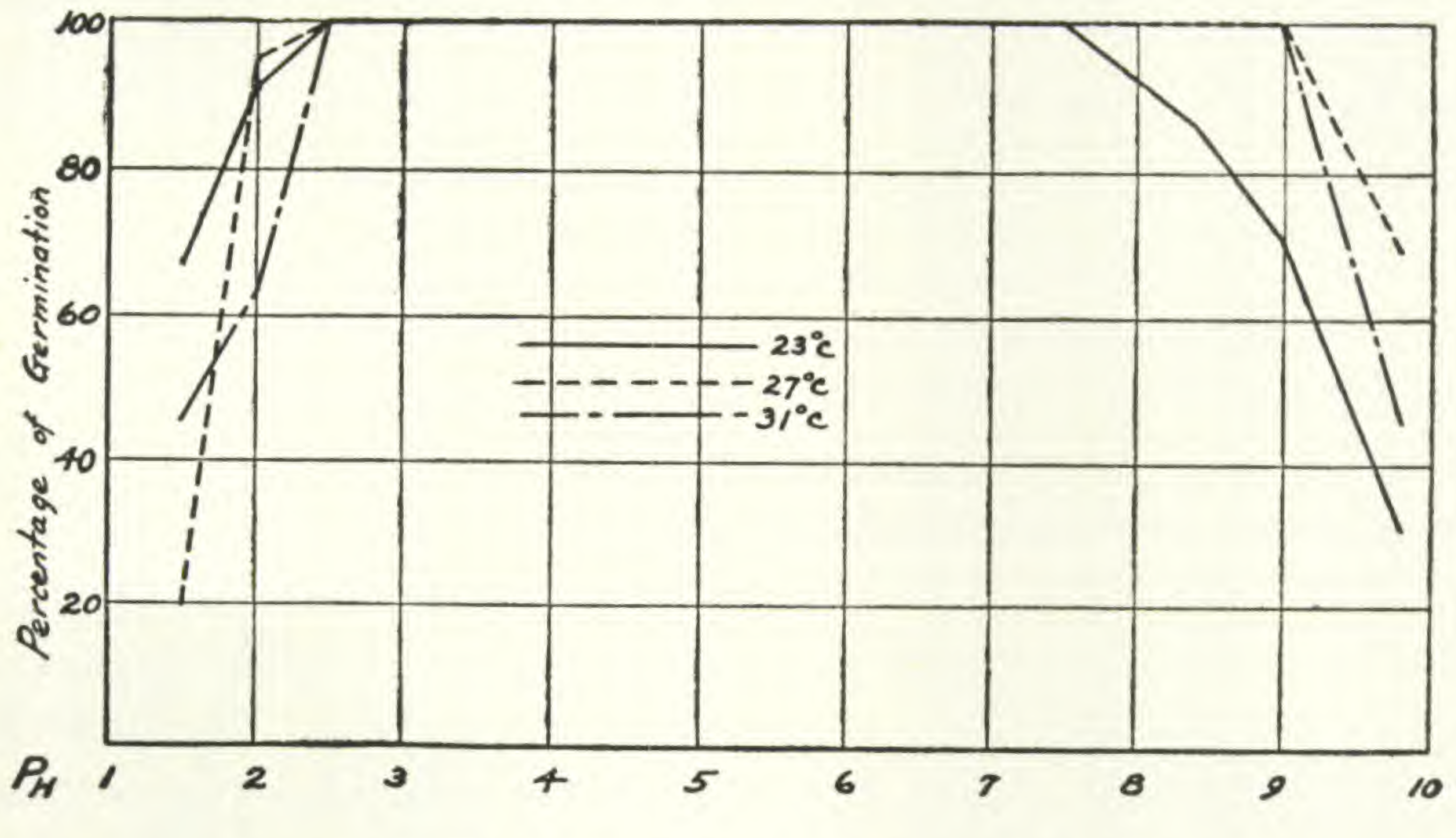

Fig. 11. *Aspergillus niger* in sugar beet decoction.

which is strikingly supported by a relatively high H-ion concentration. In various forms, notably in *Penicillium* and *Fusarium,* and under certain conditions, secondary maxima at or near the neutral point occur, and these often approach in magnitude the primary maxima on the acid side. It is not necessary, of course, to assume that the H-ion concentration most favorable for germination will also prove most favorable for the continued growth and development of the organism. In this work, it was found that germinating spores of *P. graminis* conspicuously exhibit only rudimentary germ tubes or knob-like projections

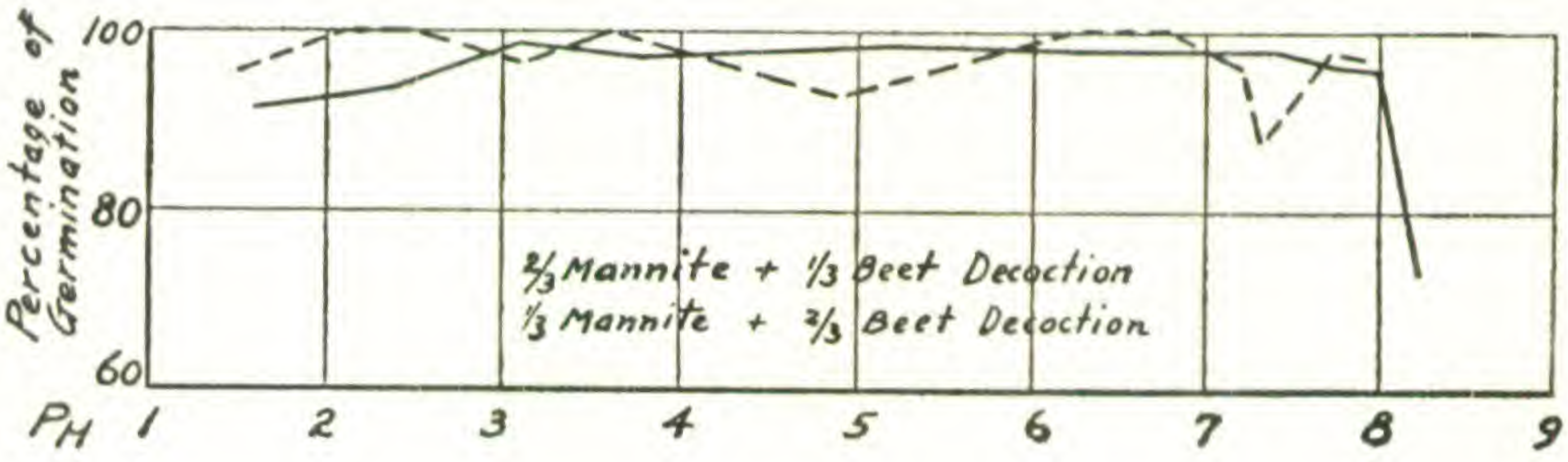

Fig. 12. *Aspergillus niger* in a medium composed of M/5 mannite and sugar beet decoction (25°C.).

———— 66⅔ per cent mannite + 33⅓ per cent sugar beet decoction.

- - - - - - - - - 33⅓ per cent mannite + 66⅔ per cent sugar beet decoction.

in the most alkaline nutrient cultures included within the favorable range and that all of the fungi show abnormal and irregularly-shaped germ tubes in the most acid cultures allowing germination, indicating, therefore, that such reactions while favorable for germination are unfavorable for growth. The germ tubes of *B. cinerea* disintegrate at P_H 2.1.

Nutrient media for pathological and bacteriological work are usually neutral or slightly acid, while my data show that increasing concentrations of hydrogen ions from neutrality to approximately P_H 3.0–4.0, in the various culture media, favorably influence the germination of the spores of *B. cinerea, A. niger, P. cyclopium, P. italicum, P. graminis, L. saepiaria,* and *Fusarium* sp. However, with increase of hydrogen-ion concentration above this zone, the germination quantities abruptly diminish and soon reach a zero value. Under conditions of moderate active alkalinity, on the other hand, relatively low percentages of germination are obtained in most cases. Some detailed discussion is needed in order to compare adequately these results and at the same time to consider the work of others.

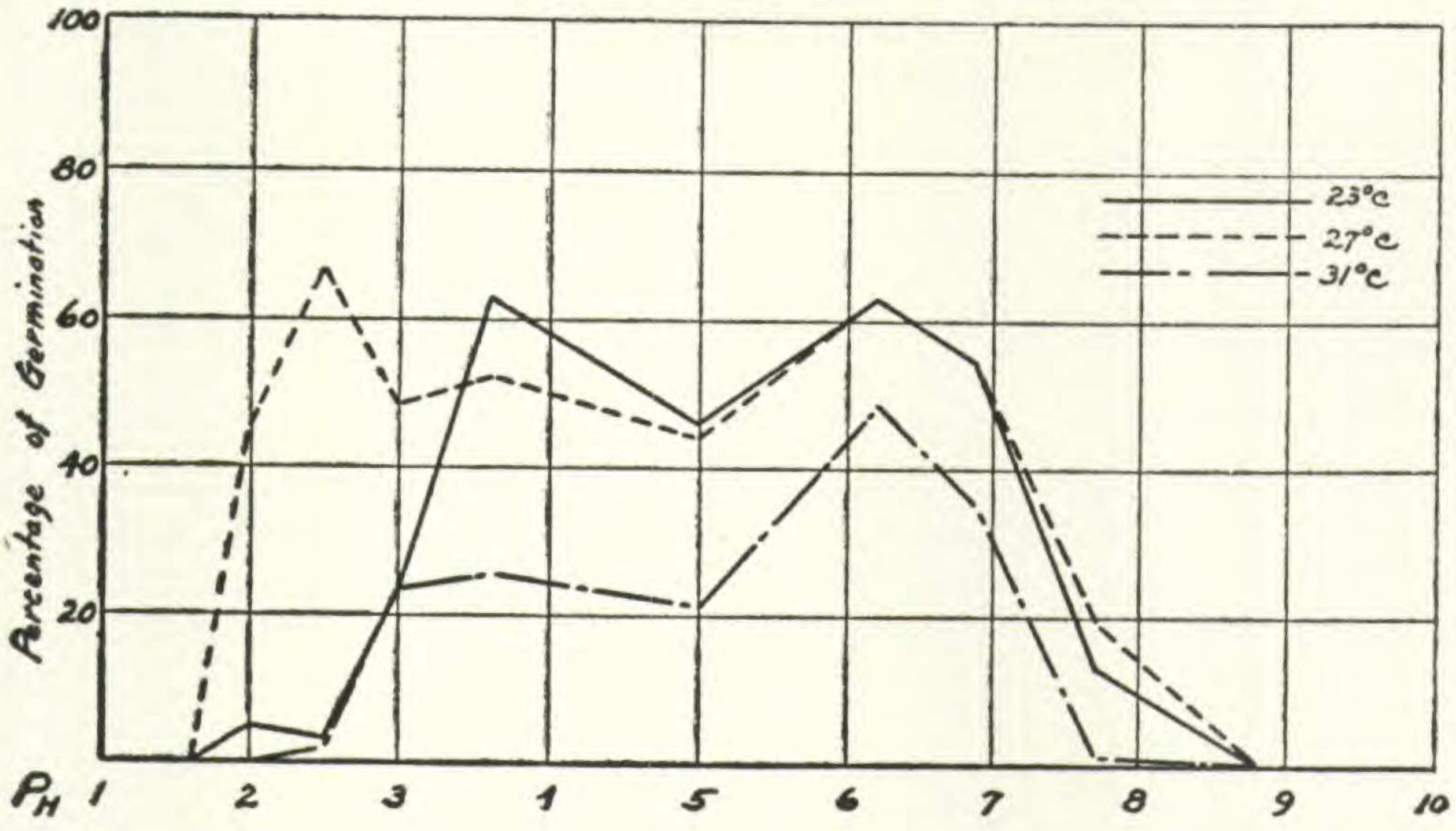

Fig. 13. *Penicillium cyclopium* in M/5 mannite solution.

From my results, *B. cinerea* may be regarded either as extremely sensitive to active alkalinity in solutions of mannite, Czapek,

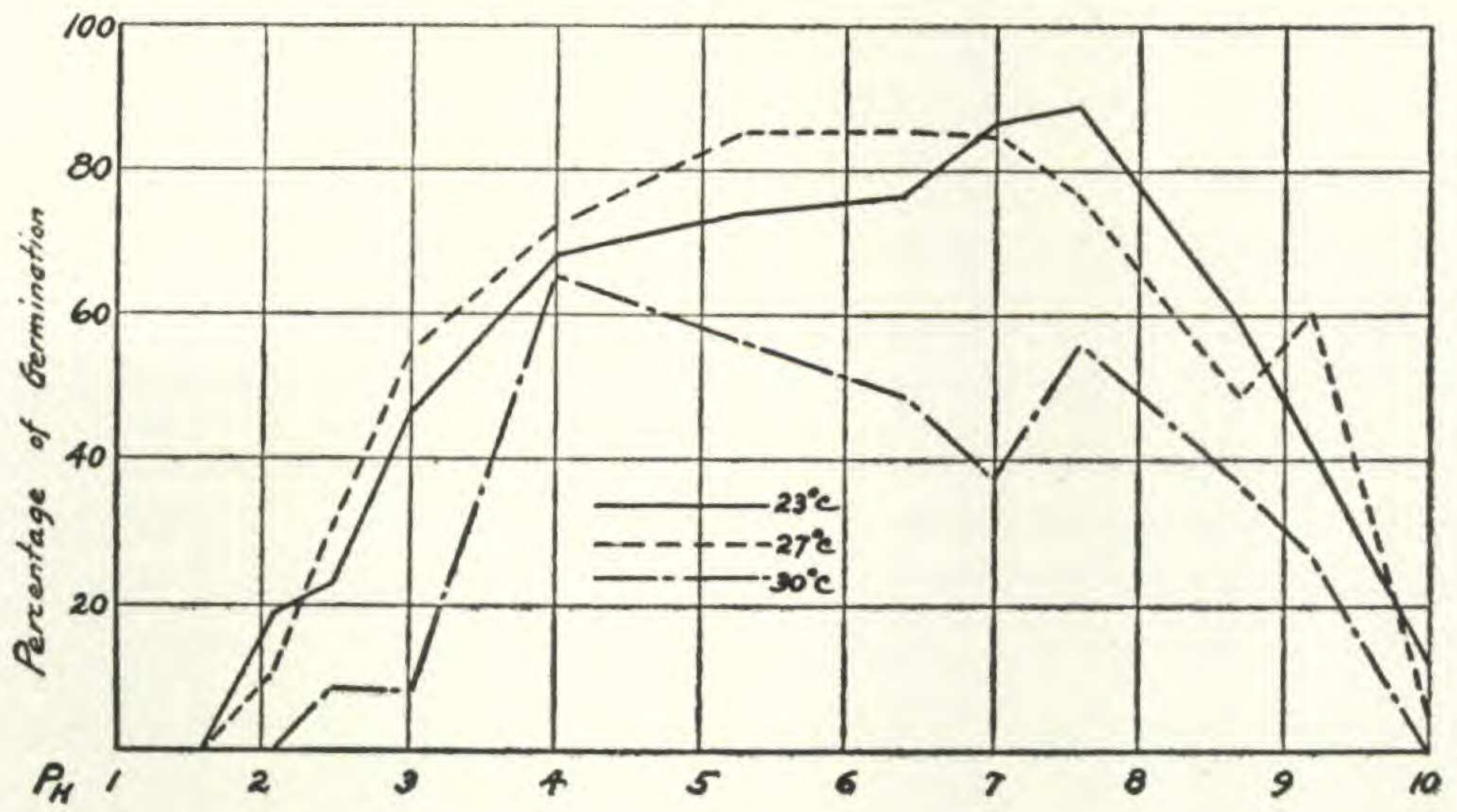

Fig. 14. *Penicillium cyclopium* in 2 per cent bacto-peptone solution.

peptone, and "water H_3PO_4 and NaOH" and to a less degree in beet decoction and "water HCl or KOH," or else as manifesting a certain dependence upon the stimulating effects of hydrogen-ion concentration in such media. Maximum germination generally occurs between P_H 2.5 and 4.0, but in the case of beet decoction the optimum zone extends from P_H 3.0 to 6.5. A secondary maximum near neutral is shown only in the case of "water HCl or KOH." The favorable range on the acid side extends more or less uniformly to P_H 2.0 in the solutions, but varies considerably in the alkaline solutions. The narrowest range of germination is exhibited in "water HCl or KOH," extending to P_H 5.5, and followed in order by solutions of mannite, P_H 6.9, Czapek, P_H 7.9, peptone, P_H 8.7, beet decoction, P_H 9.8, and "water HCl or KOH," P_H 10.0 +. The results obtained with these spores in solutions of mannite are very similar to those previously presented by the writer. It may be well to cite the concentrations of certain acids and alkalis allowing normal or almost normal development of the spores of *Botrytis vulgaris* in beet decoction, as determined by Clark ('99): HCl, N/128; HNO_3, N/256; H_2SO_4, N/64; acetic acid, N/256; monochloracetic acid, N/512; dichloracetic acid, N/256; trichloracetic acid, N/128; HCN, N/8192; KOH, N/64; and NH_4OH, N/256. He concludes that the hydroxyl group, OH, is rather more toxic to the moulds studied than ionic H.

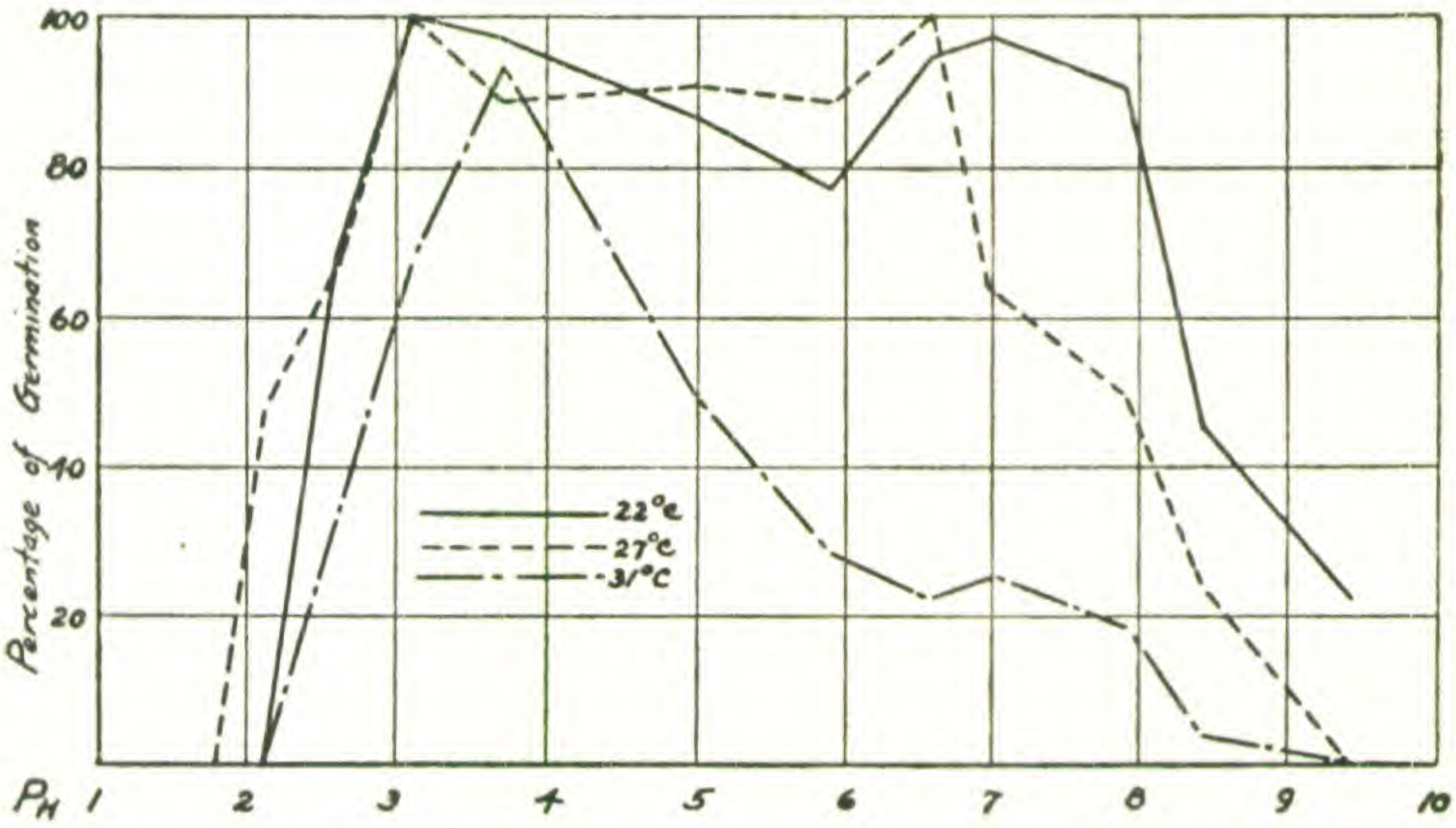

Fig. 15. *Penicillium cyclopium* in Czapek's full nutrient solution.

A. niger exhibits a relation to hydrogen-ion concentration similar to that of *B. cinerea*. Maximum germination is furnished between P_H 3.0 and 4.0 in all media except beet decoction. Here the range extends from the mentioned concentration to P_H 7.5. Low percentages of germination, except in beet decoction, occur under conditions of active alkalinity. Clark found that the spores of *Aspergillus flavus* were somewhat more resistant to the various acids and alkalis in beet decoction than were those of "*Botrytis vulgaris.*" The results presented in this paper for *A. niger* and *B. cinerea* further substantiate this relation, and the data obtained for the former in solutions of

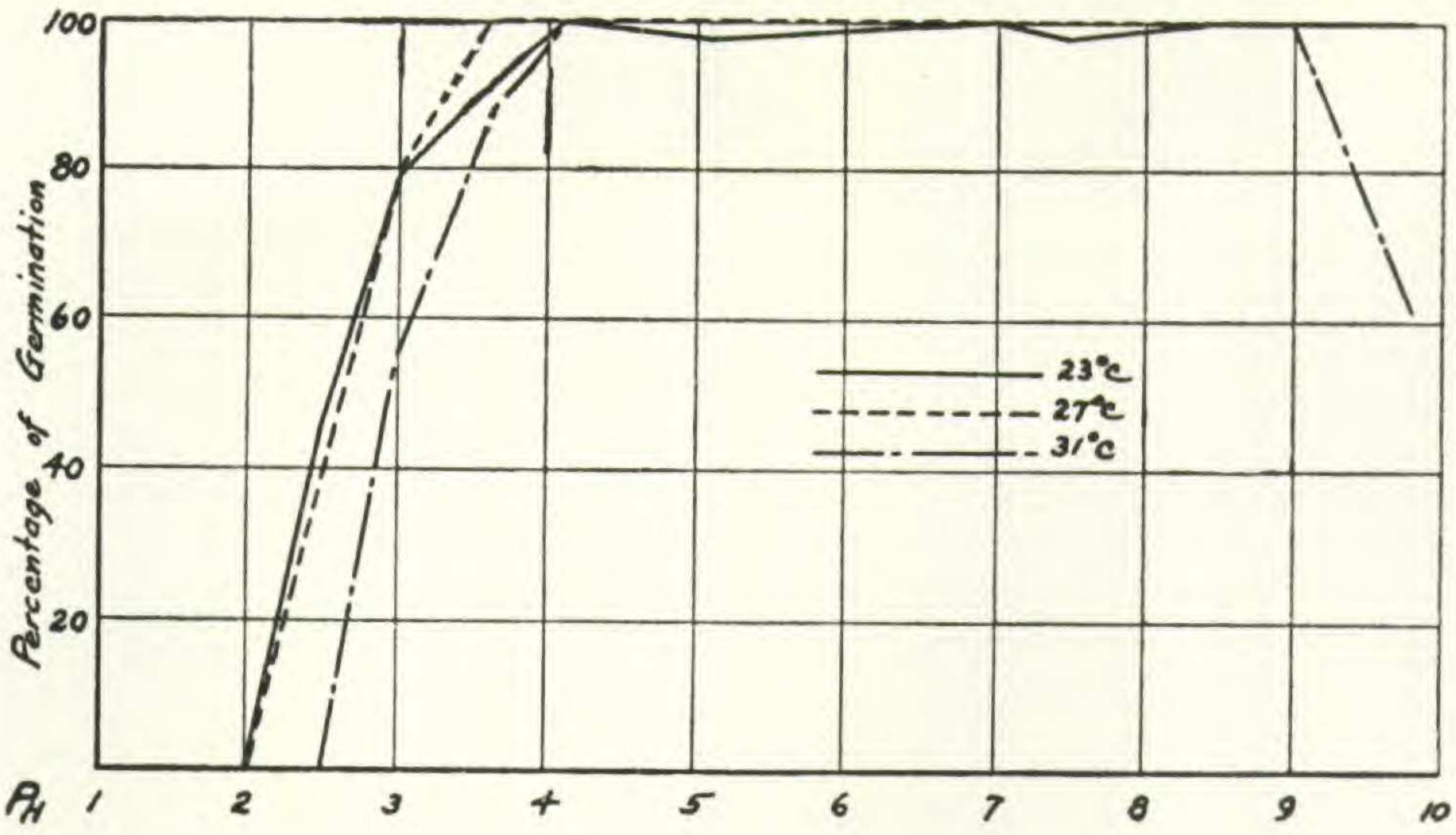

Fig. 16. *Penicillium cyclopium* in sugar beet decoction.

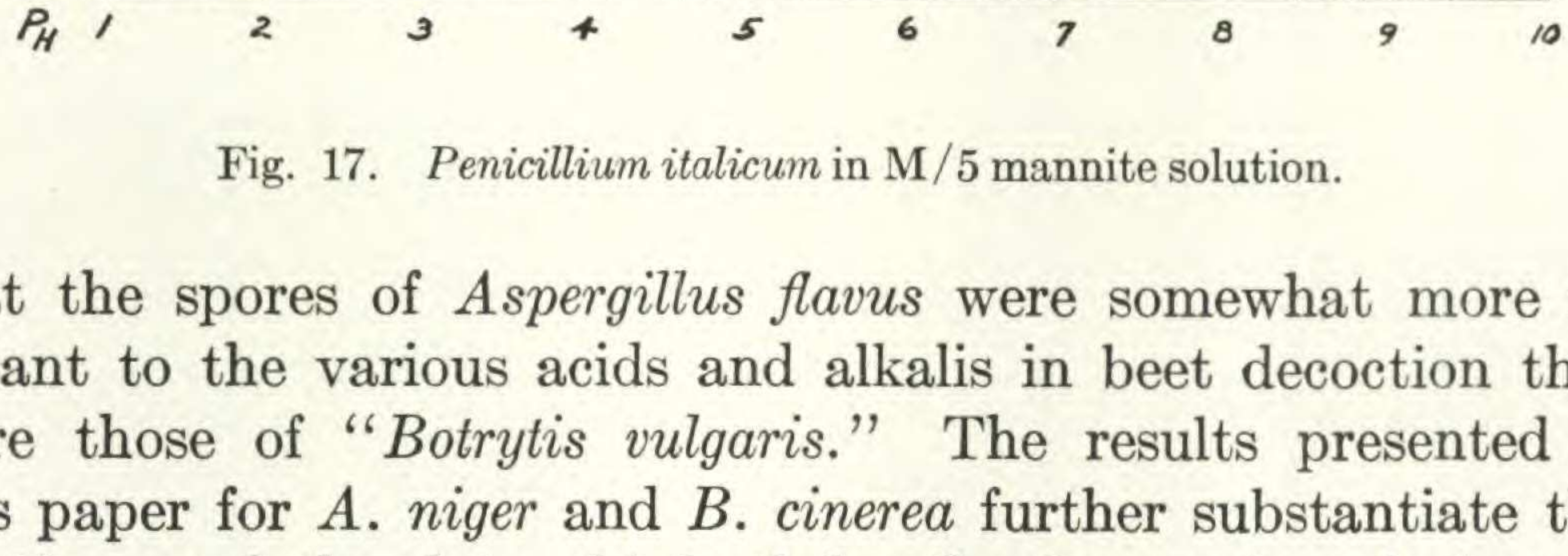

Fig. 17. *Penicillium italicum* in M/5 mannite solution.

mannite are consistent with those previously published by the writer.

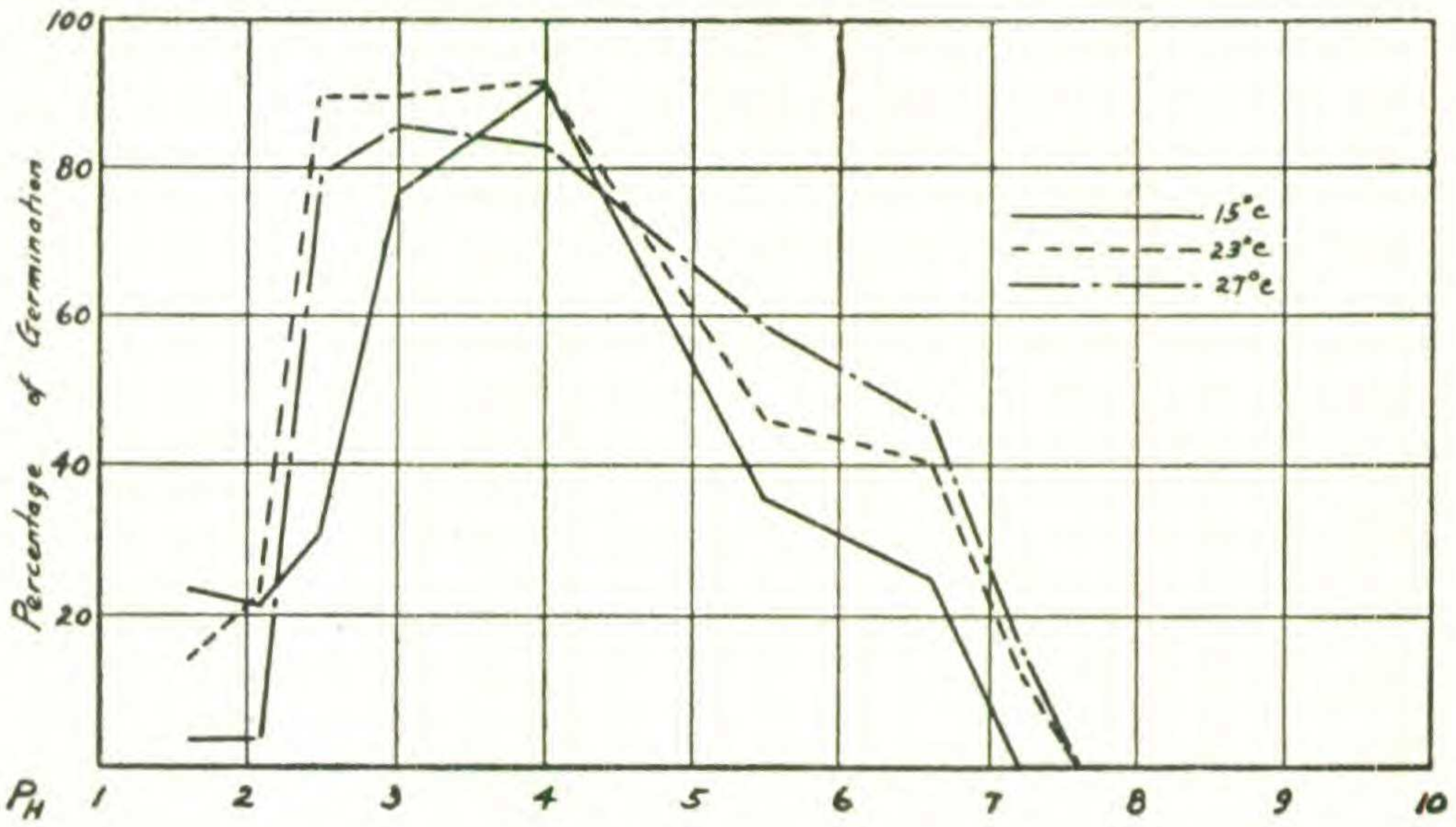

Fig. 18. *Penicillium italicum* in 2 per cent bacto-peptone solution.

The spores of *P. cyclopium* germinated under conditions very similar to those described for *B. cinerea* and *A. niger*, but a tendency is manifested by these spores to germinate more freely under neutral and slightly alkaline conditions. Two maxima, one from P_H 3.0 to 4.0 and the other about P_H 7.0, frequently appear. Of all the forms which he studied, Clark found *Penicillium glaucum* the most resistant to acids and alkalis, as well

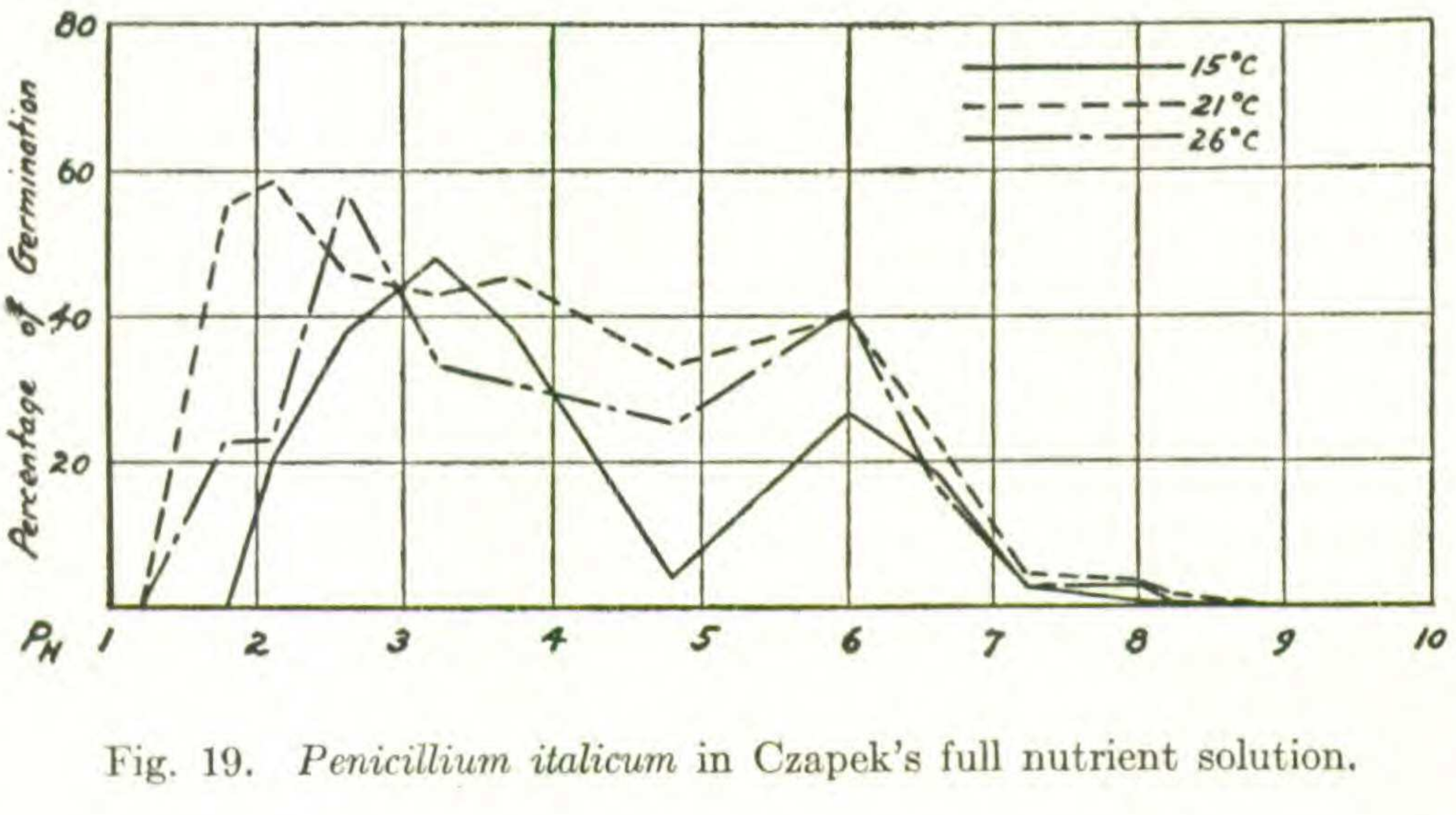

Fig. 19. *Penicillium italicum* in Czapek's full nutrient solution.

as to other poisons, the inhibiting concentrations on the whole being greater than those submitted for *B. vulgaris.* Stevens' results indicated that *Penicillium crustaceum* is more resistant to poisons in aqueous solutions than any of the other fungi studied by him. Growth occurred in N/50 HCl and H_2SO_4, while N/40 KOH and NaOH caused death. While the germination curves for *P. cyclopium* previously obtained in solutions of mannite do not agree in every detail with the curves reported in this paper for the same medium, certain features are similar.

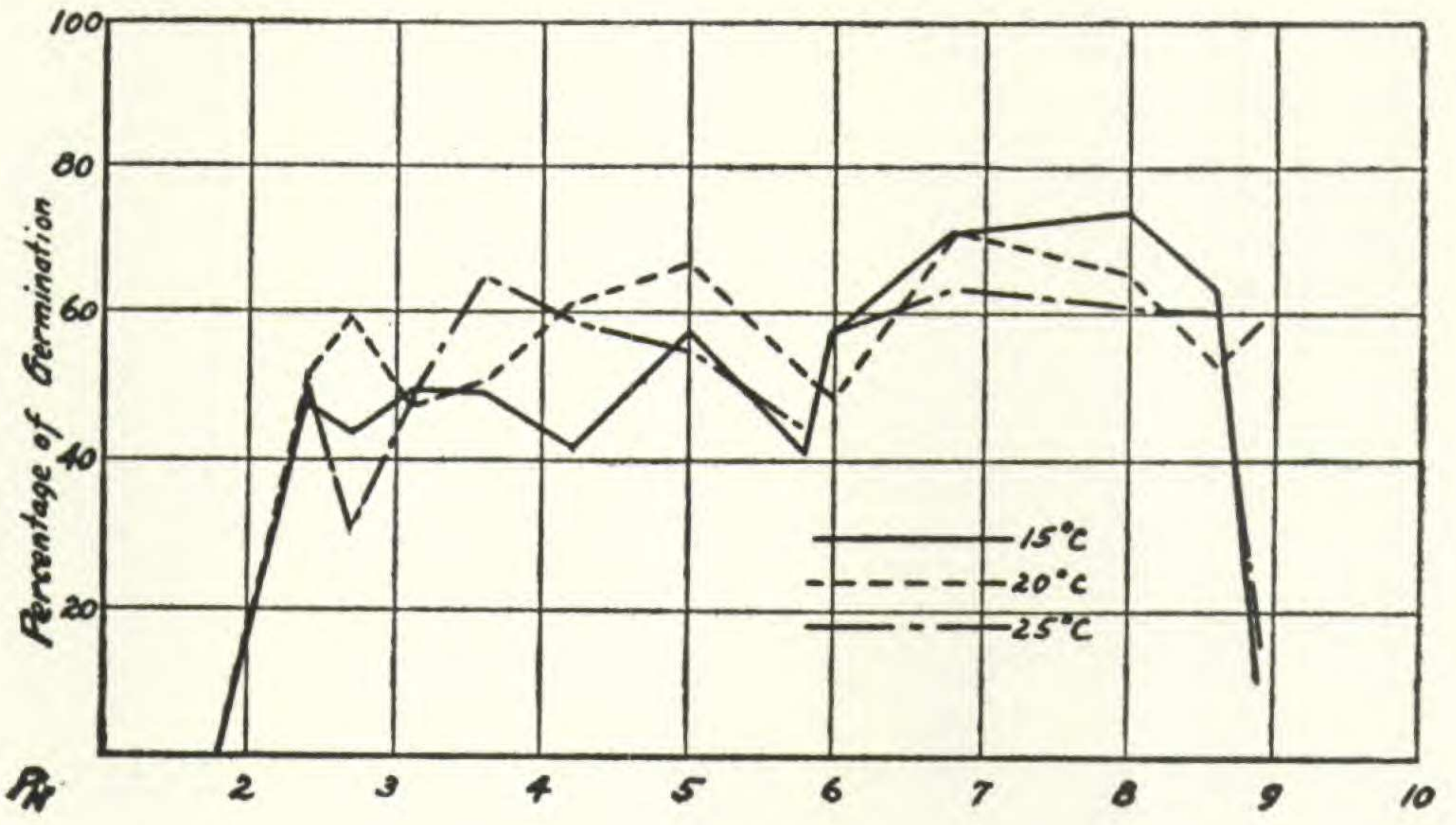

Fig. 20. *Penicillium italicum* in sugar beet decoction.

An acid reaction decidedly favors the growth of *Penicillium italicum* and under certain conditions germination is here shown to be influenced by similar conditions. The highest germination in the peptone and in the Czapek's solutions is between P_H 2.0 and 4.0 and practically no germination occurs beyond the neutral point. The data further show that germination takes place more quickly in the peptone medium that in any other, this being the only instance in which germination takes place readily after an incubation interval of 10 hours. A period of 20 hours is required in all other cases. Beet decoction, on the other hand, affords best germination in the alkaline solutions, but the difference between the highest average on the acid side and that on the alkaline side is very slight. The germ tubes in the alkaline cultures are short and stubby and give few signs of growth and development. Growth and development in the acid cultures, however, are decidedly greater. This recalls the question suggested by Duggar ('01), of media stimulating germination and not growth, and vice versa. Maximum germination, as determined from my experiments, occurs within zones rather than at definite points.

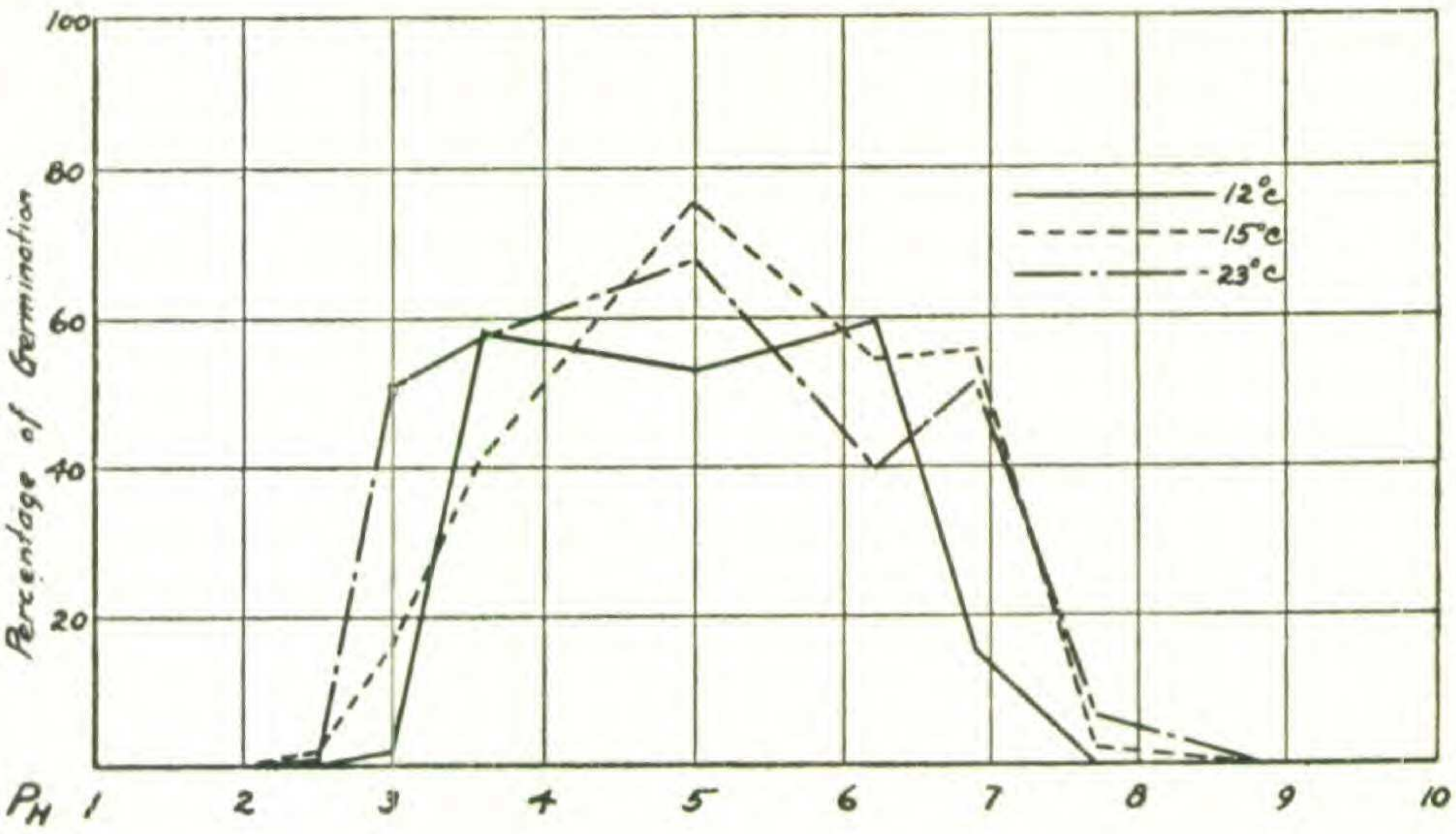

Fig. 21. *Puccinia graminis* in M/5 mannite solution.

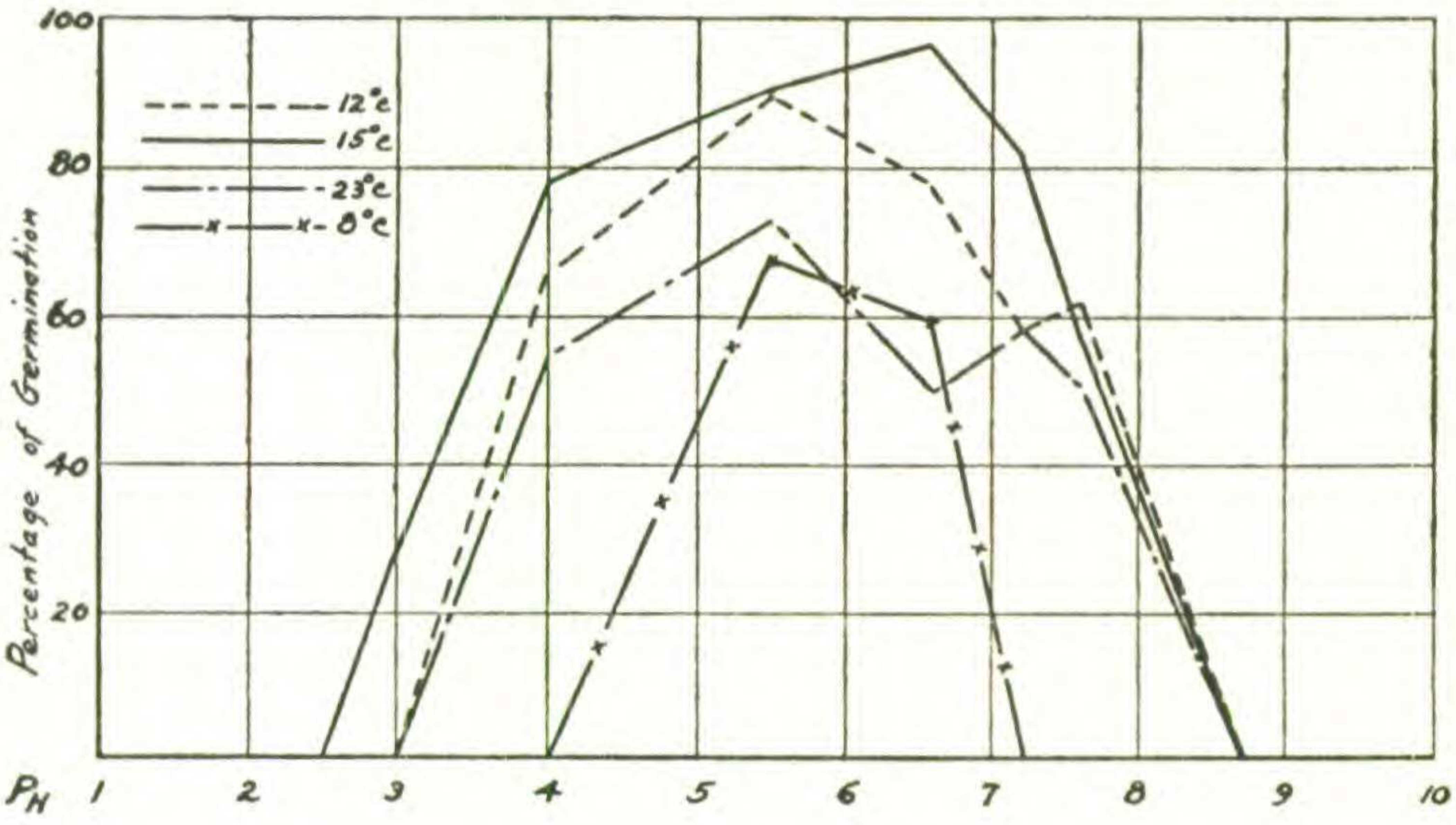

Fig. 22. *Puccinia graminis* in 2 per cent bacto-peptone solution.

L. saepiaria, like certain other species included within the acid group, proved very sensitive to an alkaline reaction, and while the limiting concentration on the acid side is somewhat lower than in the case of other included forms, these fungi are more or less similar in behavior. The results obtained in the various culture solutions are consistent and comparable. Certain stimulating effects, it will be noted, are exhibited by the neutral cultures of the beet decoction, but the favorable range in this medium does not expand as it does for certain of the other fungi. Meacham ('18) obtained inhibition of growth of *L. saepiaria* at approximately P_H 1.7 in synthetic media and malt-extract, and it is of interest to note that he frequently obtained a maximum of growth at about P_H 3.0, which approaches very closely the hydrogen-ion concentration in the mannite, Czapek, peptone, and beet decoction solutions which afford maximum germination.

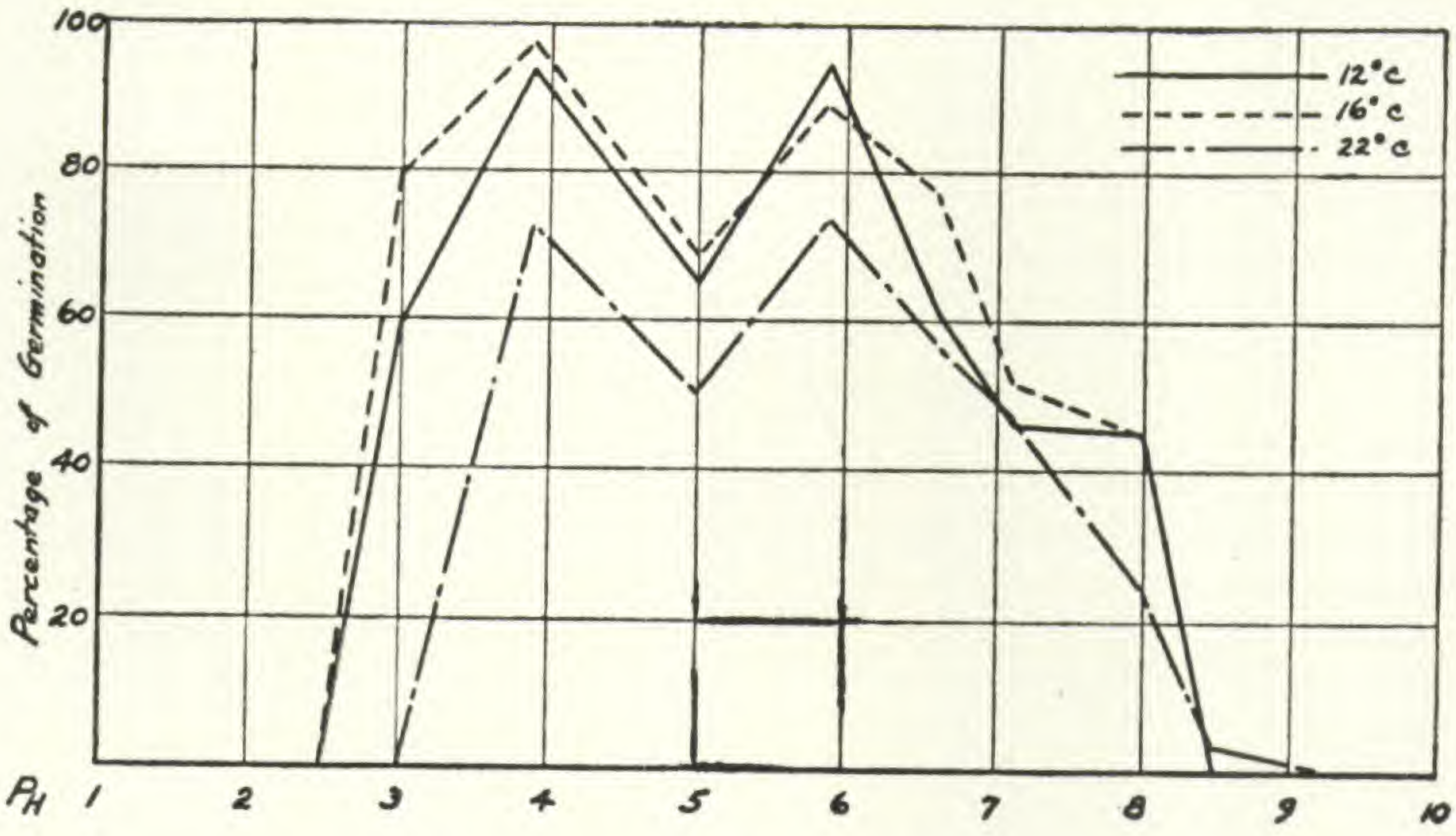

Fig. 23. *Puccinia graminis* in Czapek's full nutrient solution.

The uredospores of *Puccinia graminis* do not germinate, on the whole, in solutions with as high hydrogen-ion concentrations as some of the other fungi employed, the favorable range terminating between P_H 2.5 and 3.0 as compared with P_H 1.5 to 2.0 in other cases. Relatively low percentages of germination are obtained under conditions of active alkalinity, except in the case of "water HCl or KOH," and such percentages vary materially with the medium. In "water HCl or KOH," germination occurs freely at P_H 9.4, a hydrogen-ion concentration producing inhibition of germination with the other media.

The expansion of favorable range on the alkaline side in water is striking and analogous to what resulted with *Botrytis*. A slightly acid or neutral reaction, on the whole, decidedly stimulates the germination of these spores.

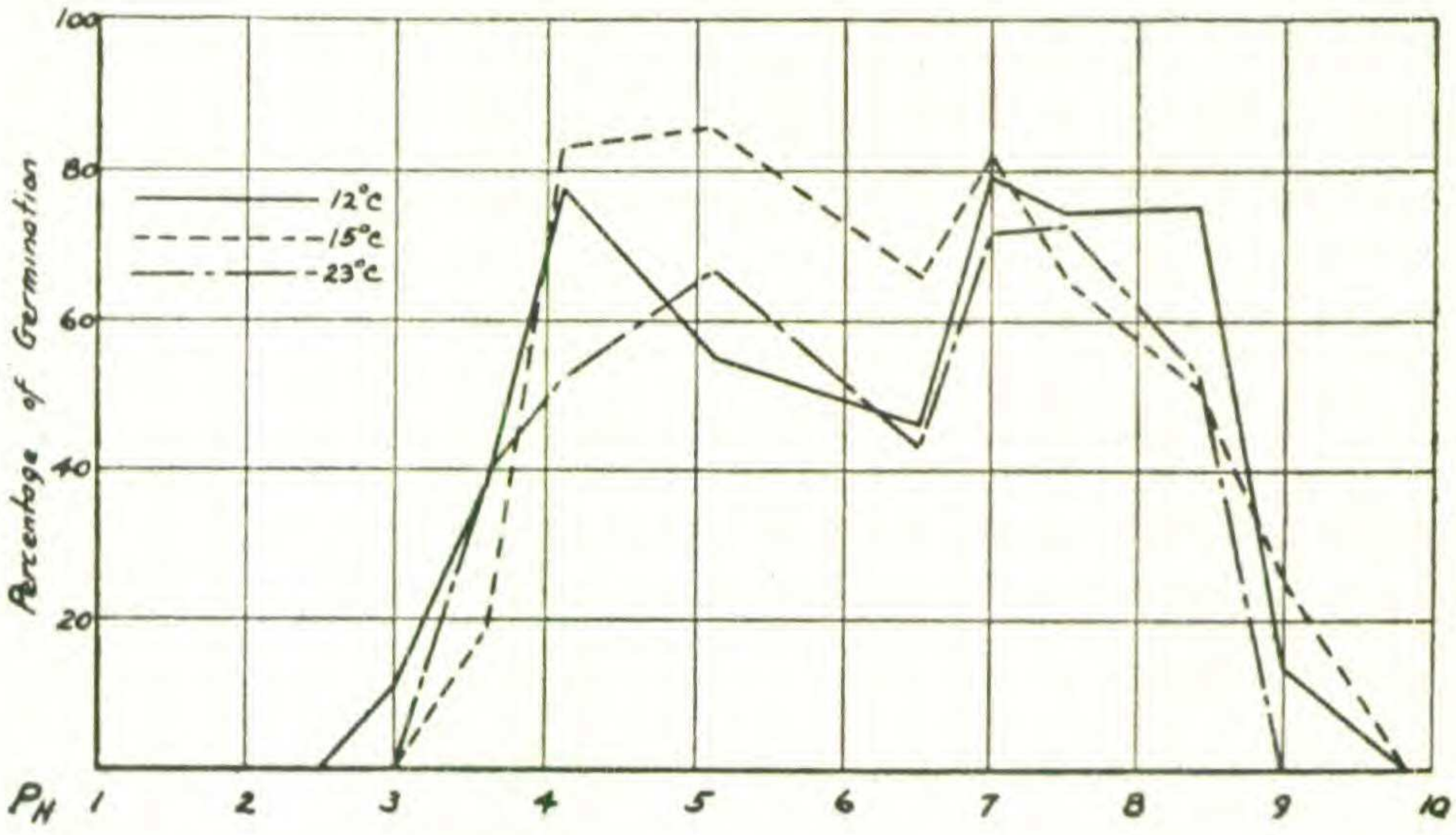

Fig. 24. *Puccinia graminis* in sugar beet decoction.

Of the forms studied, *Fusarium* sp. is the only one that decidedly responds to an alkaline medium. This form, moreover, exhibits the widest range of germination with respect to the reaction of the medium, extending from P_H 2.0 to P_H 10.0 +, the most alkaline culture. Maxima generally appear about P_H 3.0–4.0 and near P_H 7.0.

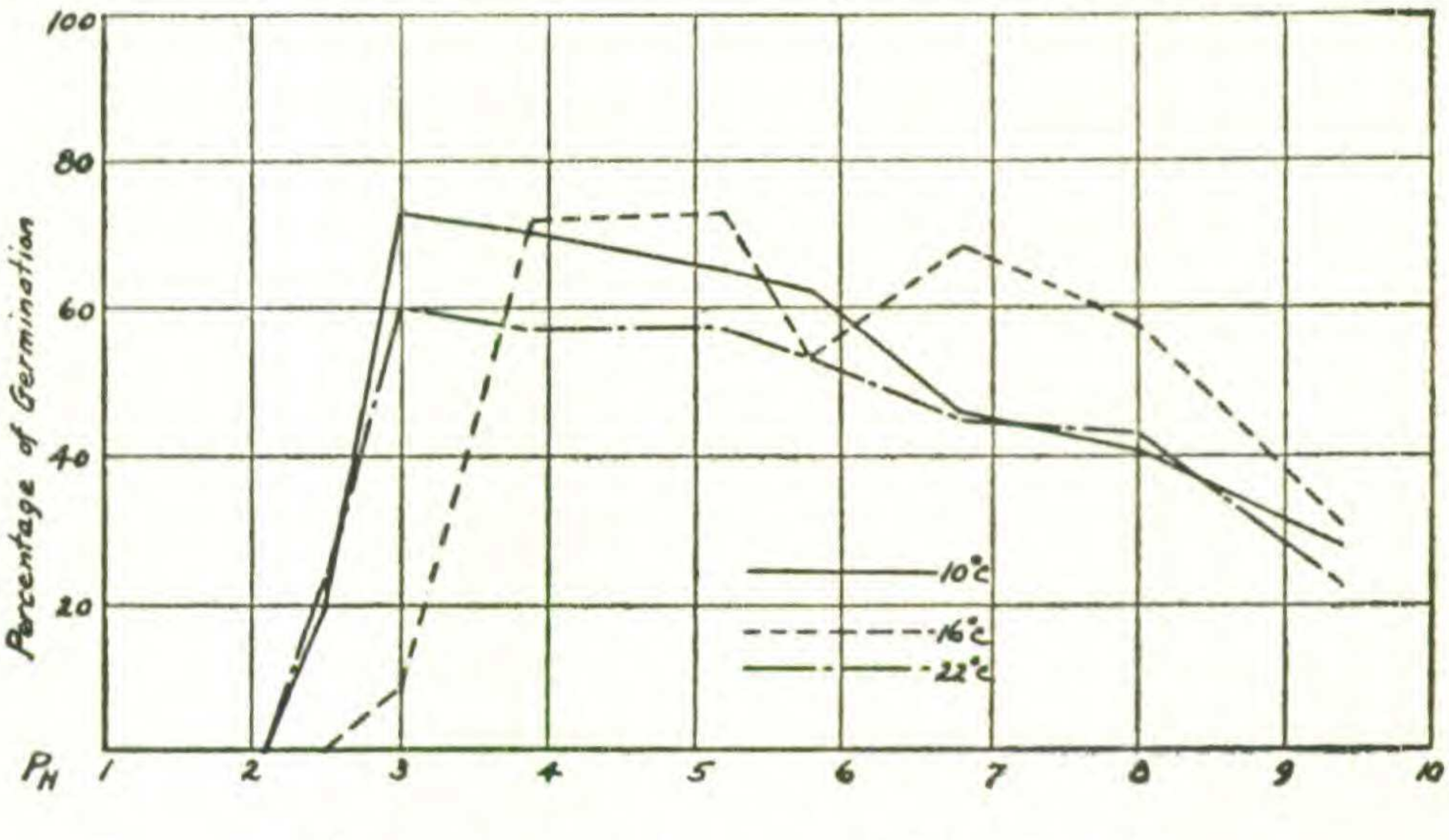

Fig. 25. *Puccinia graminis* in "water HCl or KOH."

The spores of *C. Gossypii* are very variable in germination and give no definite and characteristic curve of germination.

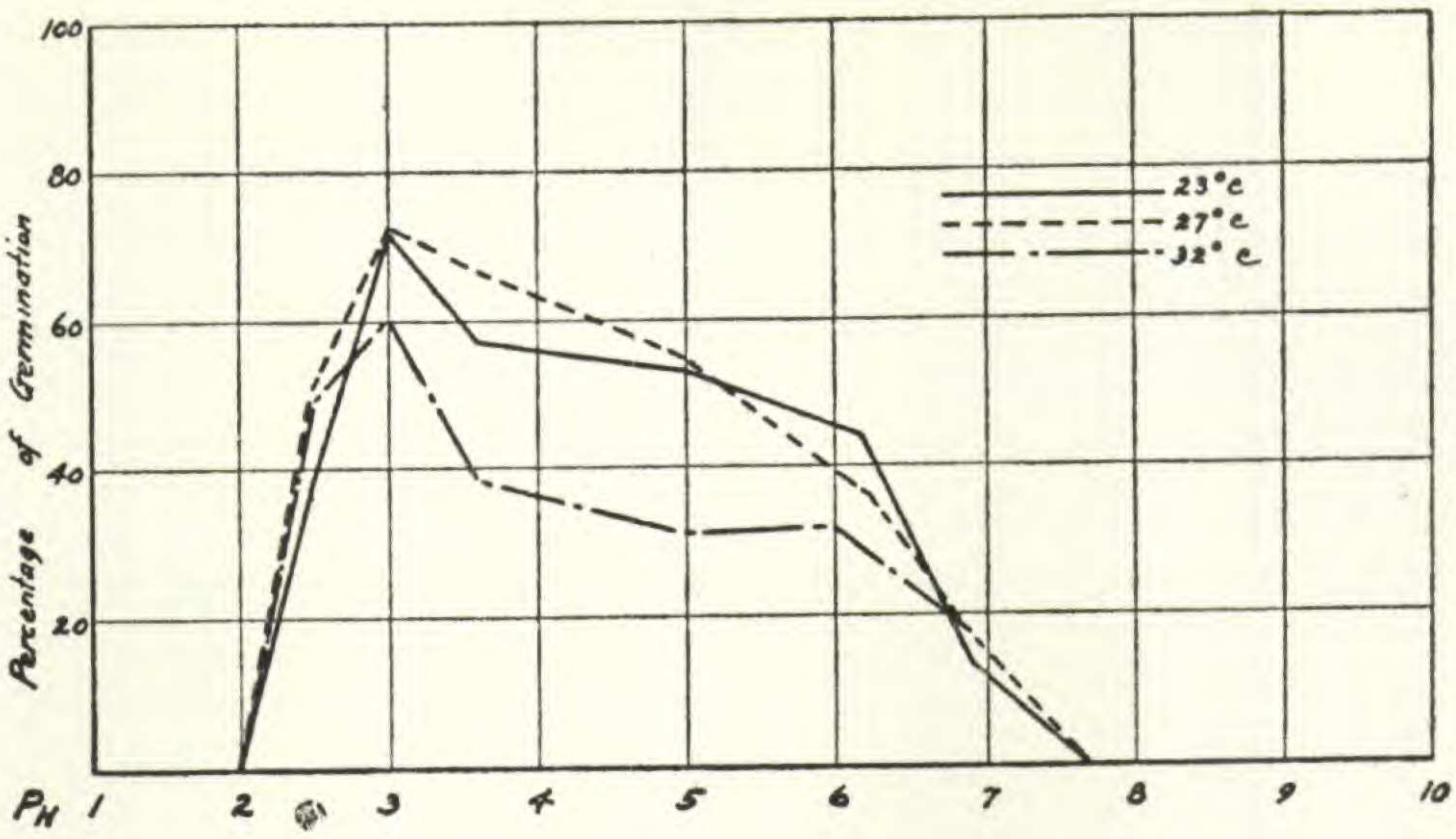

Fig. 26. *Lenzites saepiaria* in M/5 mannite solution.

Beyond emphasizing the favorable effects of an alkaline or slightly acid medium, nothing can be said. In the case of the peptone solutions, the usual type of germination did not occur; that is, not with the production of germ tubes. On the contrary, secondary spores were formed at the ends of the mature spores, and the data in peptone solution for this organism represent percentage quantities of this phenomenon. The remaining

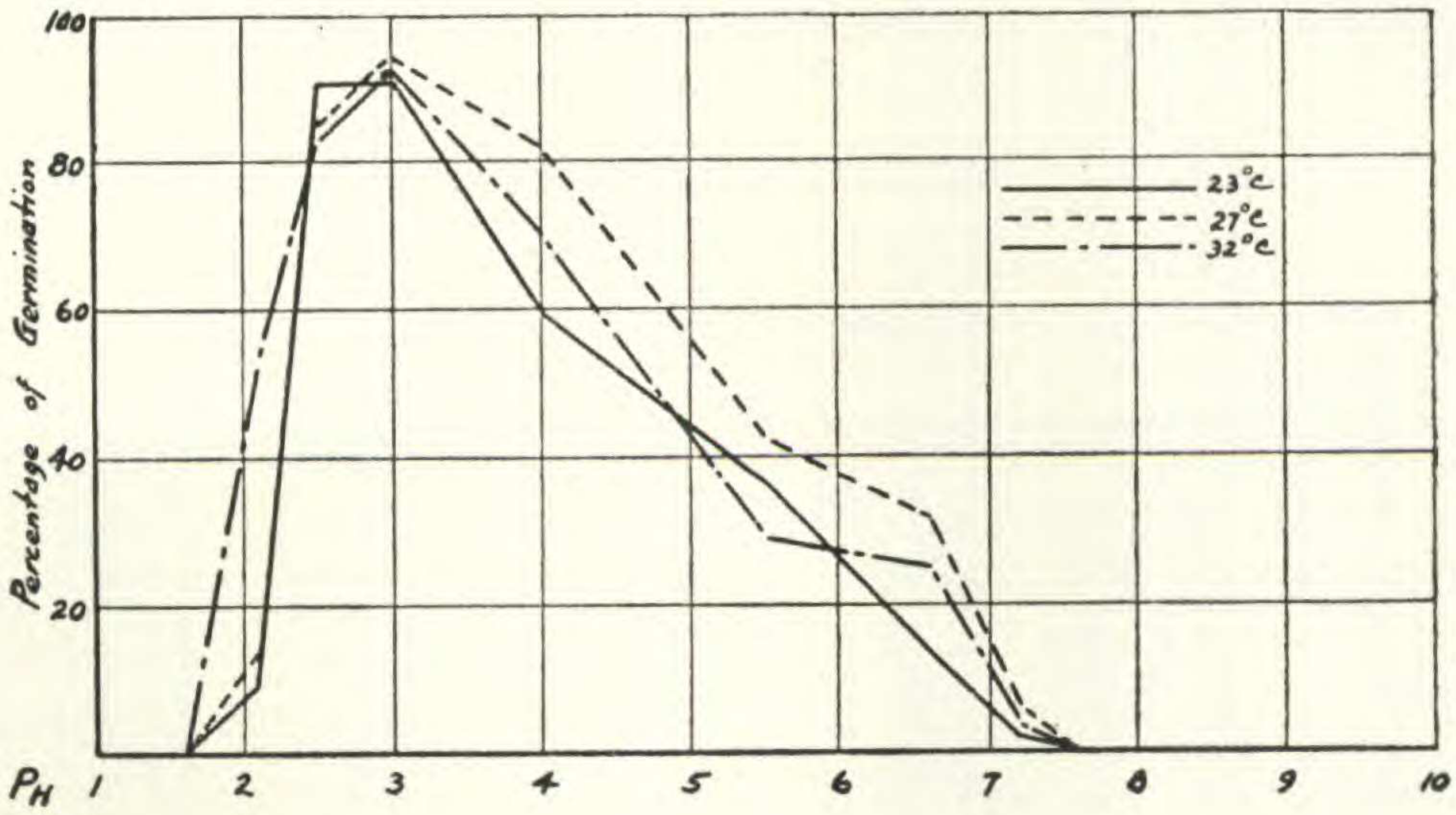

Fig. 27. *Lenzites saepiaria* in 2 per cent bacto-peptone solution.

culture solutions, on the other hand, gave normal germination. For 10 hours at 23° and 27° C., the secondary spores were still attached; at 31° C., however, they were detached. When incubated for 20 hours at each of the temperatures, the spores were detached and numerous. According to Stoneman ('98), "the formation of the so-called secondary spores or buds which are common to *Gloeosporium*, *Colletotrichum*, *Volutella*, and *Vermicularia* is not a constant character, but may be absent throughout the entire cycle of development of a species, or may be forced in the same species by a lack of nourishment."

The spores of *Ustilago Avenae* from the material available gave such relatively low percentages of germination with the described method of technique that this organism was discarded. However, series were conducted in mannite solutions at 15°, 19°, and 25° C. In general, germination ranges from P_H 2.4 to 8.2, with a maximum at P_H 6.2. Sporidia occur most abundantly from P_H 5.4 to 7.0 and decrease in number with increase in departure from either side of this zone.

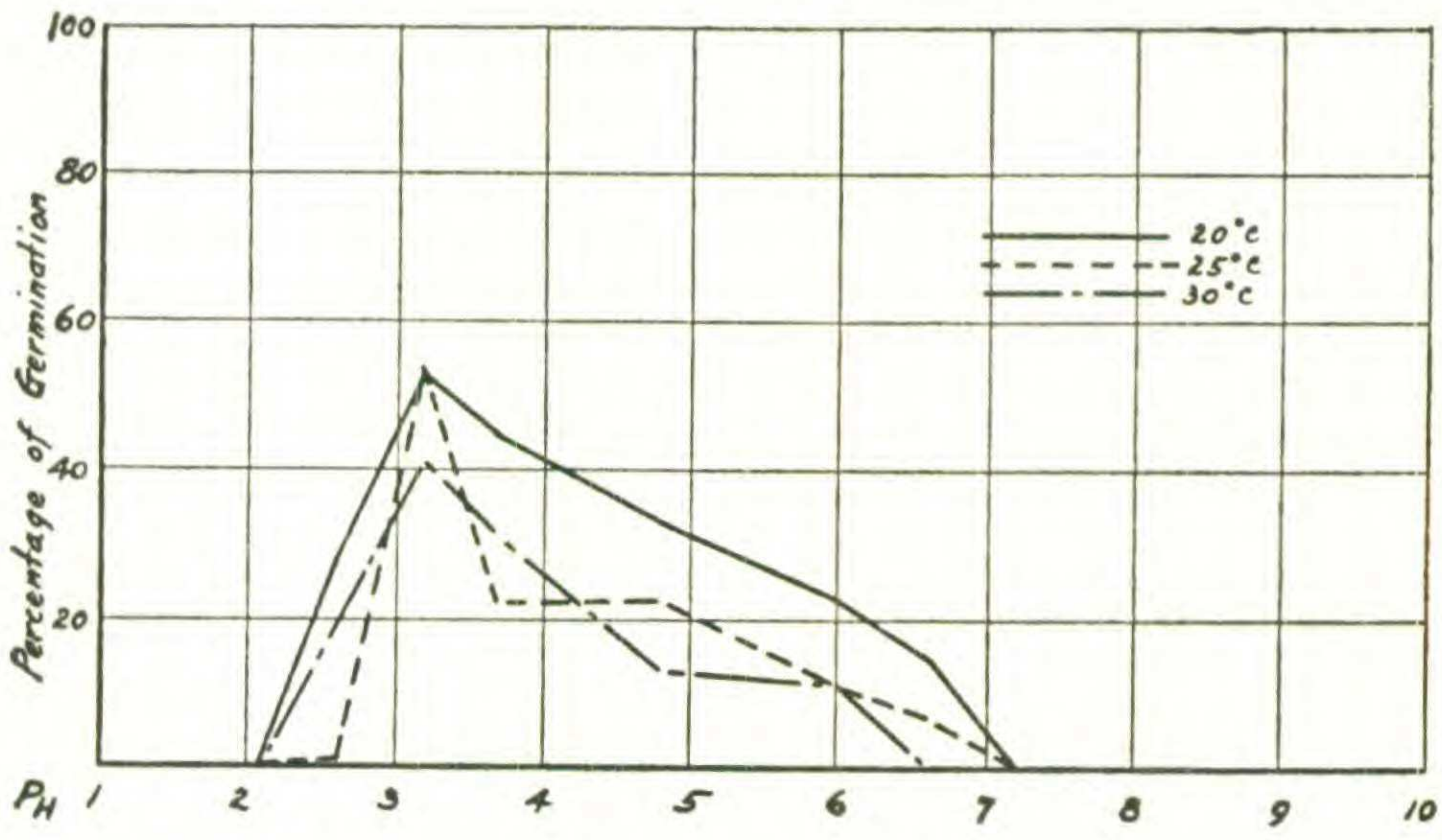

Fig. 28. *Lenzites saepiaria* in Czapek's full nutrient solution.

Repeated endeavors have also been made to study conidial germination with certain of the powdery mildews, but all attempts have been without avail. No germination whatever has thus far been obtained with *Sphaerotheca pannosa* at different temperatures when the conidia were placed either in the hanging drop or in film cultures of solutions of mannite, sucrose, beet decoction, tap water, and distilled water. Previous subjection of the spores for various time intervals to low or freezing temperatures also failed to stimulate germination.

Marked differences in requirements for germination are shown by the spores of fungi. Some are capable of germinating in moist air or in water, while others are capable of germinating only in the presence of a nutrient solution or special stimulus. The percentages of germination, in many cases, depend largely upon the direct food value of the medium; that is, the most perfect food affords the best germination. Certain of the fungi, however, germinate well in sugar solutions and best in plant infusions or decoctions. Such cases, then, may be classed as food stimuli. Organic acids are generally regarded as feeble stimuli for the germination and growth of fungi, but here again the matter is vitally associated with the question of toxicity. Taylor ('17) determined the concentrations of a few organic and inorganic acids necessary to check the growth of various organisms. His data led him to conclude that there is a great variation in specificity in the relation of such acids to different organisms.

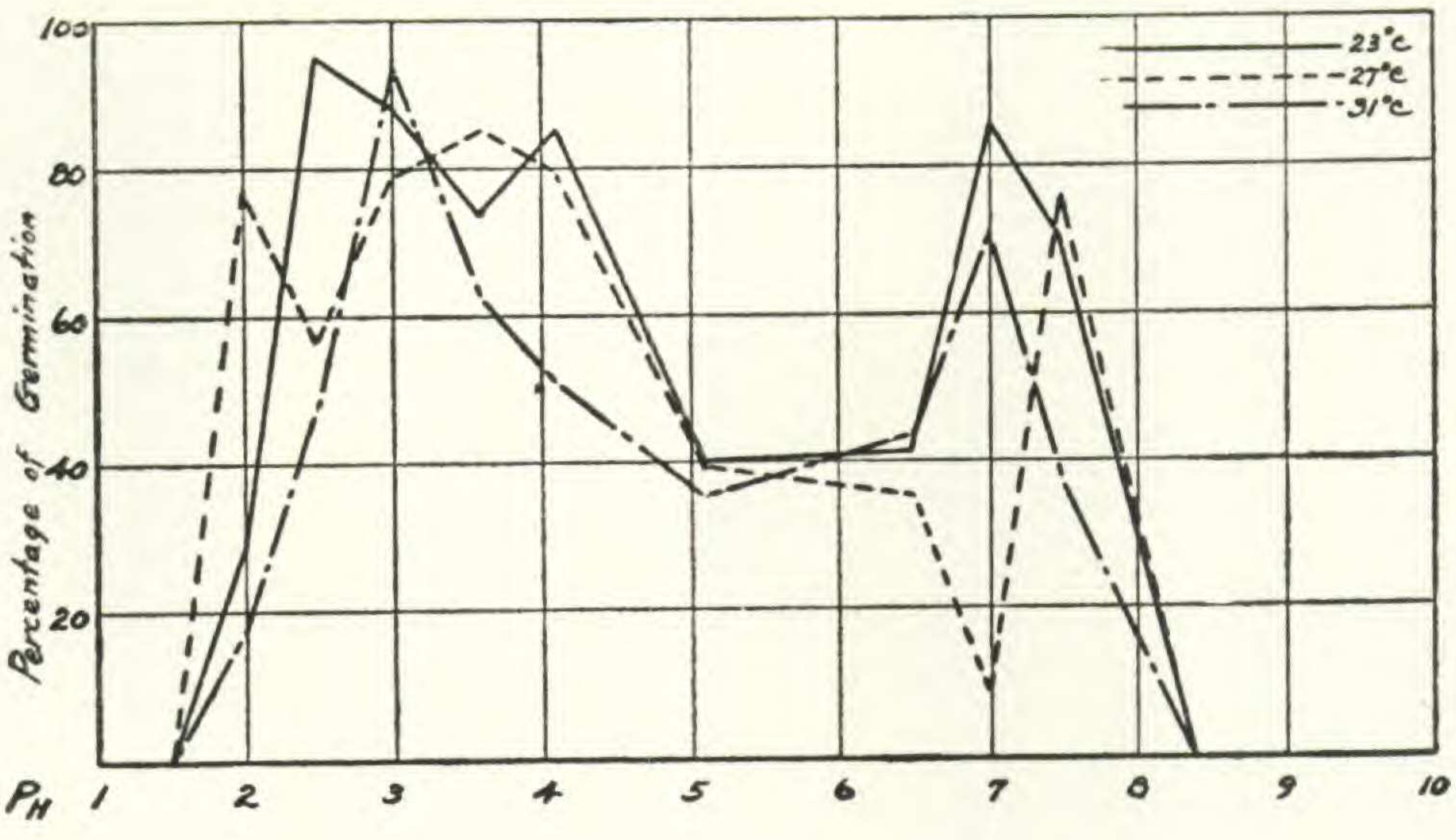

Fig. 29. *Lenzites saepiaria* in sugar beet decoction.

Brooks ('06) found that the deleterious action of $CuSO_4$, HNO_3, and H_2SO_4 in beet decoction medium upon the germination and development of certain fungous spores was least at a provisional optimum temperature and that it increased very rapidly with rise in temperature above such an optimum. The effects of the three chemicals, however, were very different, and spores inhibited by cold were not greatly injured when exposed to harmful agents.

Temperature variations such as were employed in the experiments here reported exert little or no influence upon the curves of germination, but it must be remembered that the temperature range was relatively narrow. This aspect of the problem will be considered later.

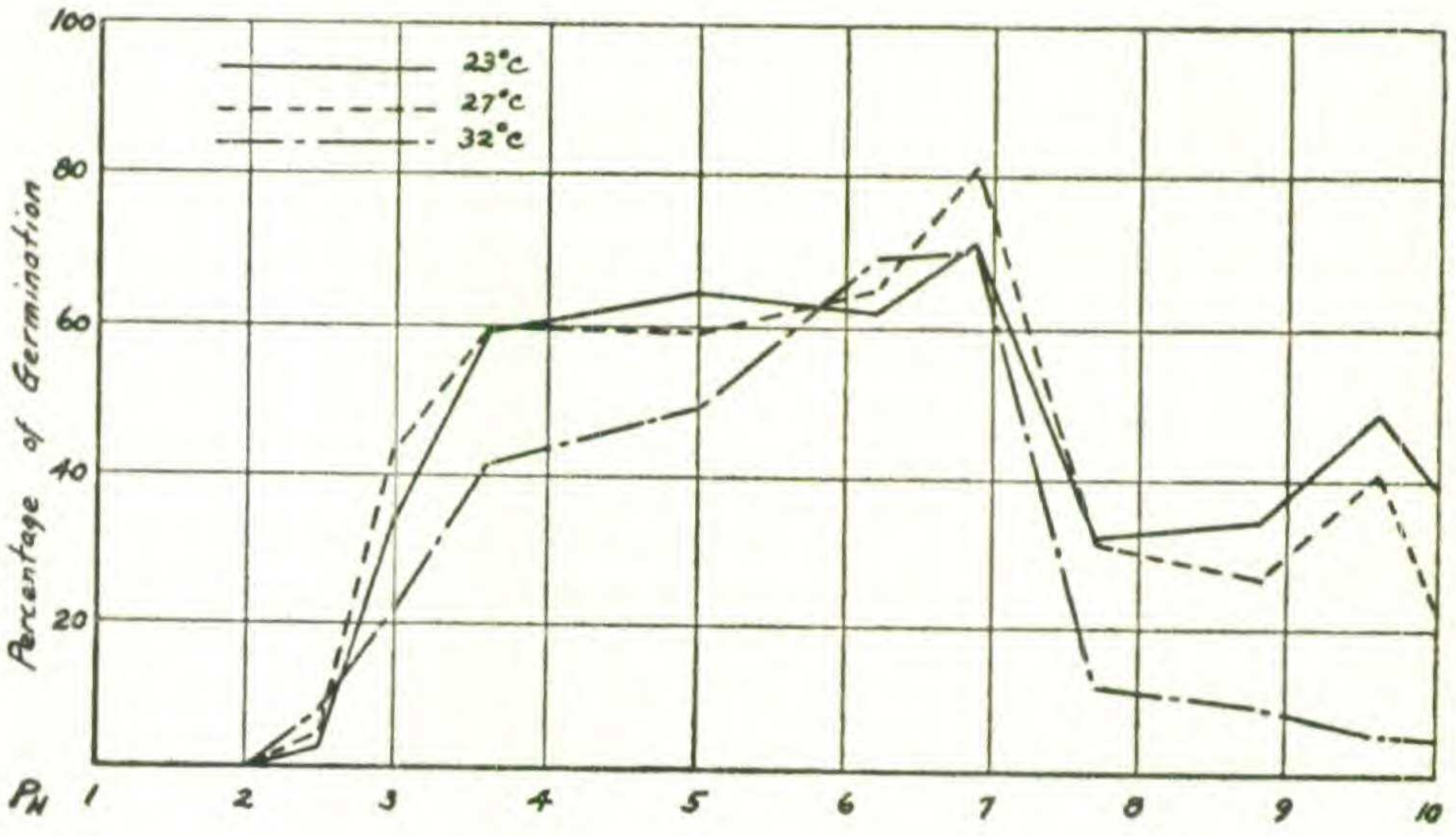

Fig. 30. *Fusarium* sp. in M/5 mannite solution

The effect of the hydrogen-ion concentration of the medium as influenced by certain types of nutrition, or the chemical composition of the nutrient medium, is shown by the results presented in this paper to be of extreme importance. Inasmuch as a detailed consideration has been devoted to these points for the individual fungi, it is necessary to mention only a few of the striking features. The results with the various media are comparable, yet it is difficult to make general statements that would be applicable in all cases.

"Water H_3PO_4 and NaOH" followed by solutions of mannite, undoubtedly gives the lowest percentages and the smallest range of germination. The conditions in the two solutions are very comparable; that is, the individual cultures contain equal quantities of H_3PO_4 and increasing quantities of NaOH, resulting therefore in the formation of NaH_2PO_4 and Na_2HPO_4 at P_H 4.5 and P_H 9.2 respectively. No Na ions are contained in the most acid culture and these ions increase in number with decrease in H-ion concentration. It is impossible, however, to vary the H-ion concentration without varying the concentration of some other ion or ions at the same time, even though such variations may be insignificant.

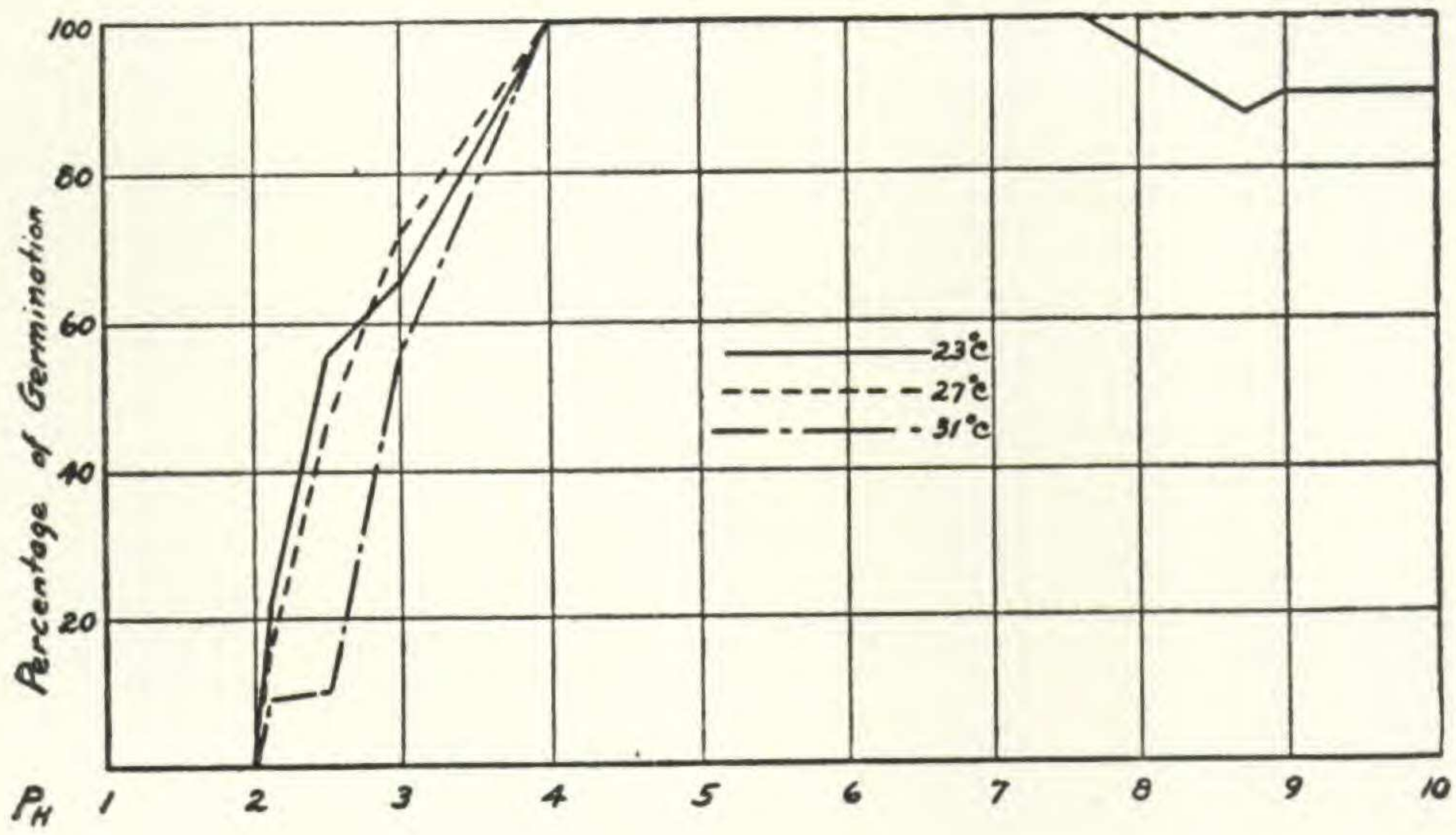

Fig. 31. *Fusarium* sp. in 2 per cent bacto-peptone solution.

Solutions of bacto-peptone give very similar results to solutions of mannite, except that germination is better, that the range is slightly more extended on the alkaline side, and that maximum germination frequently occurs in zones rather than at definite points. The reaction of the peptone solutions, as well as Czapek's, beet decoction, and "water HCl or KOH," is varied on the acid side by additions of HCl and on the alkaline side by additions of KOH. Disregarding the variation in H-ion concentration, the acid cultures thus contain increasing amounts of Cl ions and the alkaline cultures increasing amounts of K ions.

Czapek's full nutrient solution generally exhibits germination ranges similar to those afforded by the peptone solutions, but the curves of germination in the Czapek differ decidedly from those developed in the peptone. Invariably two maxima occur: one between P_H 3.0 and 4.0 and the other between P_H 6.0 and 7.0, and these maxima present more or less definite peaks in the curve. It is interesting to note that there is frequently a decided decrease of germination quantities at or near P_H 5.0. This minimum within the favorable range occurs in the culture containing the normal Czapek full nutrient solution without additions of either acid or alkali.

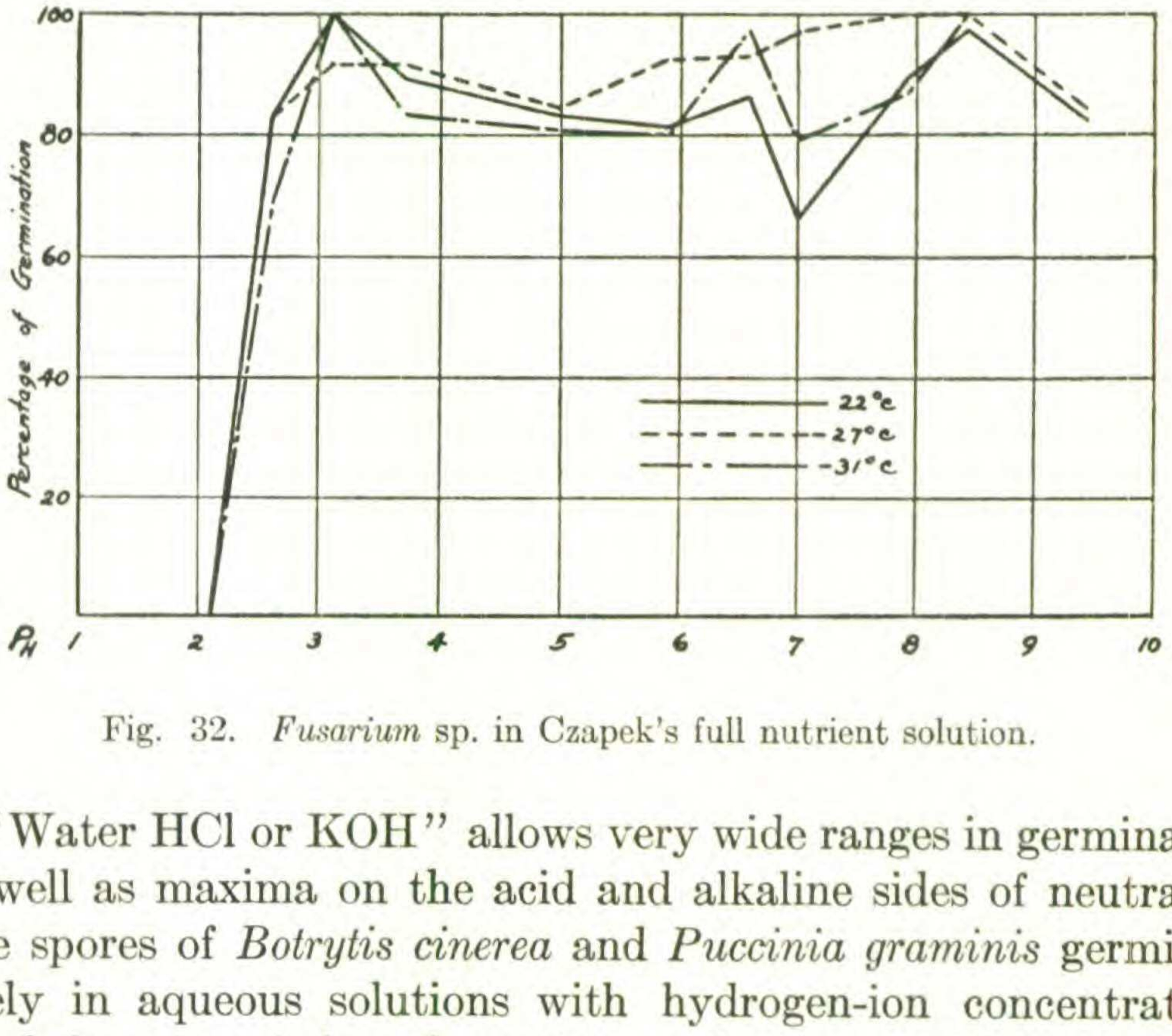

Fig. 32. *Fusarium* sp. in Czapek's full nutrient solution.

"Water HCl or KOH" allows very wide ranges in germination as well as maxima on the acid and alkaline sides of neutrality. The spores of *Botrytis cinerea* and *Puccinia graminis* germinate freely in aqueous solutions with hydrogen-ion concentrations which in most of the other culture solutions would have caused inhibition of germination.

Beet decoction, it has been shown, furnishes highest percentages and the widest range of germination as related to hydrogen-ion concentration for all of the spores employed in this investigation. Germination occurs freely under conditions of active alkalinity in this medium, and hydrogen-ion concentrations producing inhibition of germination in the other media allow good germination in this medium. The maxima generally manifest themselves within relatively wide zones, and perfect or almost perfect germination frequently continues from extreme acidity to extreme alkalinity, that is, within the limits of the experiments. From these facts, it would seem that the beet decoction possesses some stimulating substance or complex set of conditions, either or both of which are absent in the synthetic culture media, and the belief is further confirmed by several

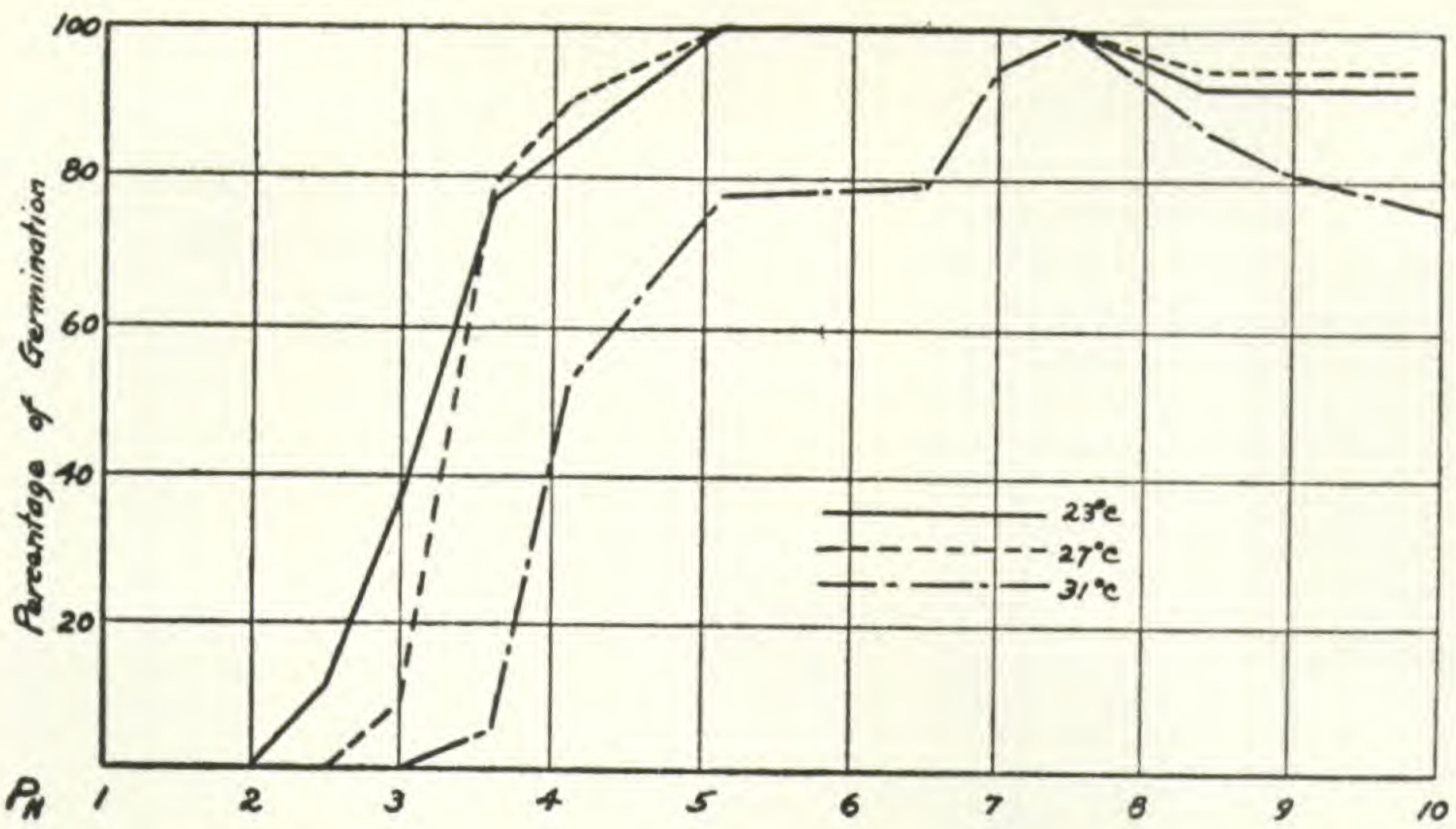

Fig. 33. *Fusarium* sp. in sugar beet decoction.

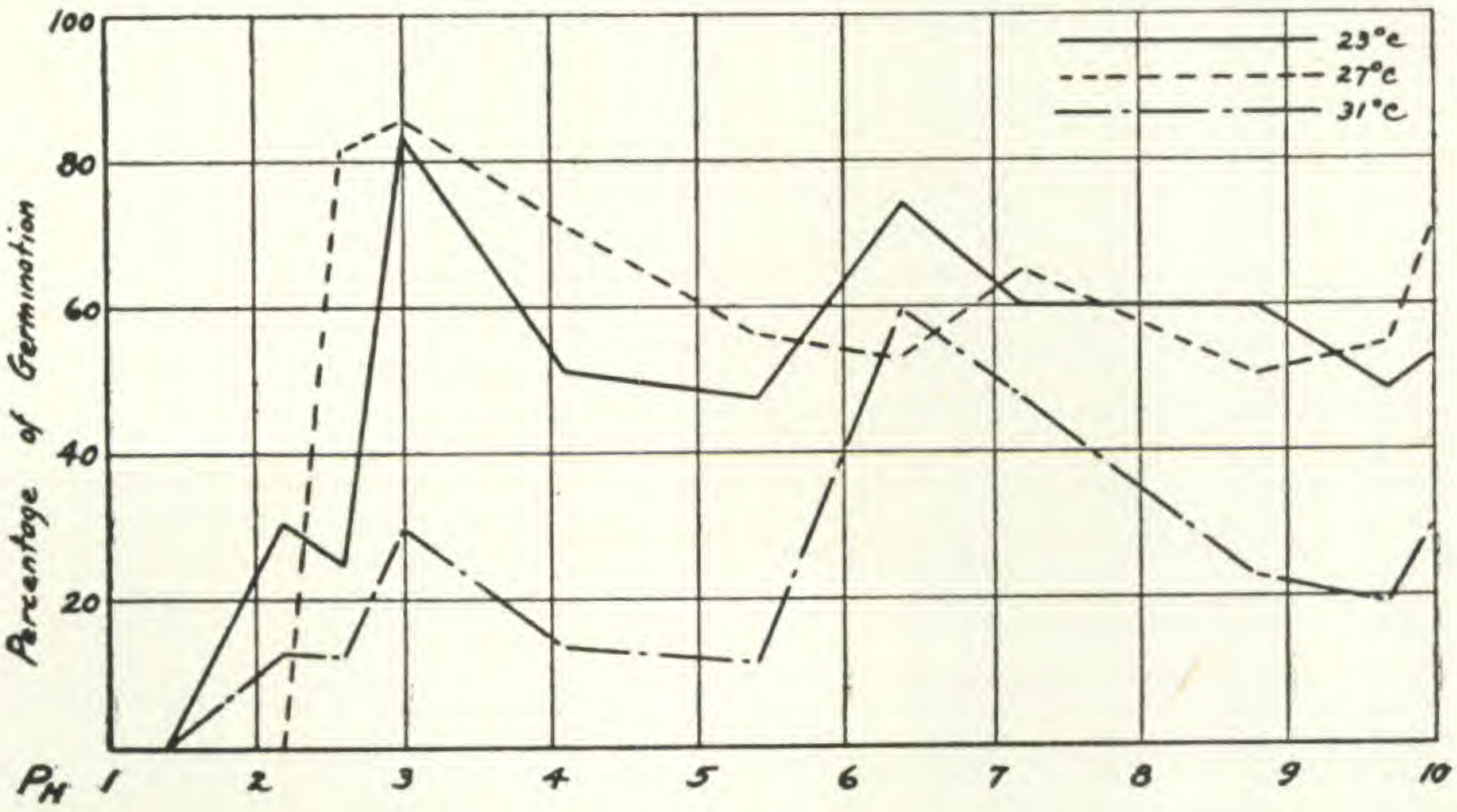

Fig. 34. *Fusarium* sp. in "water HCl or KOH."

experiments reported in this paper. Various additions of beet decoction to solutions of mannite invariably allow stimulation of germination with *A. niger* and *B. cinerea.* In both cases the germination quantities and the range of favorable germination approach those offered by the beet decoction alone and practically no resemblance to those furnished by the mannite solutions is recognizable. In the case of *B. cinerea,* the degree of stimulation near the alkaline extreme varies directly with the quantity of added beet decoction, but no such relation seems to exist in the case of *A. niger.* Duggar ('01) reports a similar stimulation of certain fungous spores in plant decoctions or infusions. He obtained various small percentages of germination of *Coprinus fimetarius* in different vegetable decoctions, but otherwise no germination. *Coprinus micaceus* gave little or no germination in all solutions containing no plant decoction, but furnished perfect germination in bean and dung decoctions. Since plant decoctions are such excellent growth media, it would seem that the stimulus to germination would be a food stimulus. Duggar designed and conducted experiments to determine the stimulus in such cases, and, while this most complicated matter is still unsolved, his statement is as applicable to-day as it was originally, namely: "If the stimulus is that of food, it must be considered in the class of peculiar foods."

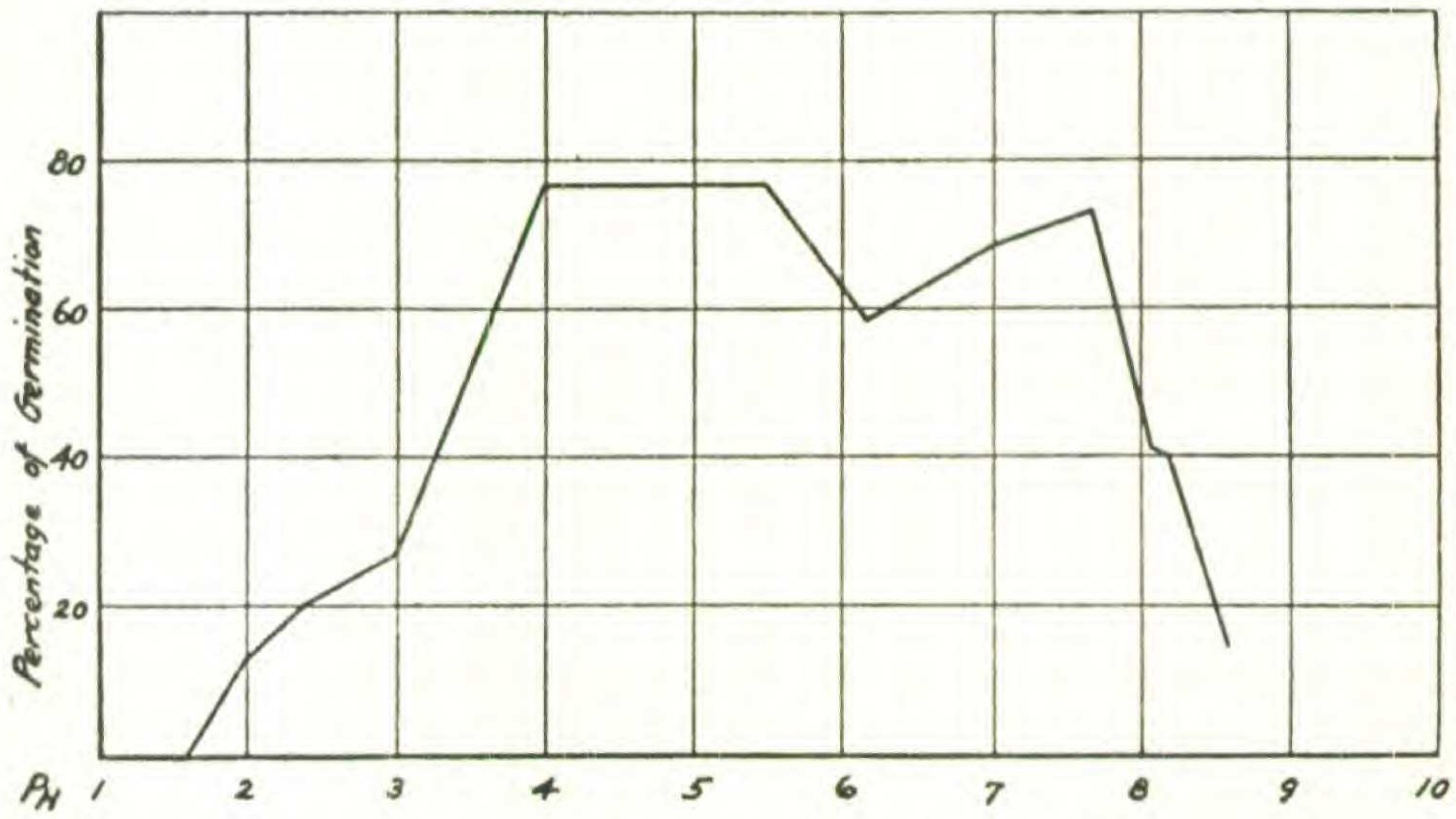

Fig. 35. *Fusarium* sp. in "water H_3PO_4 and NaOH" (25°C.).

The titration curves of the liquid culture media, fig. 1, show that "water HCl or KOH" has the least buffer action and that beet decoction has very little more. These facts, then, would seem to offer an explanation of the expanded favorable range under conditions of active alkalinity, with such solutions on the basis of buffer effect or change in reaction of the medium during germination. In this connection, Itano and Neil ('19) have recently shown that the spores of *Bacillus subtilis* germinate

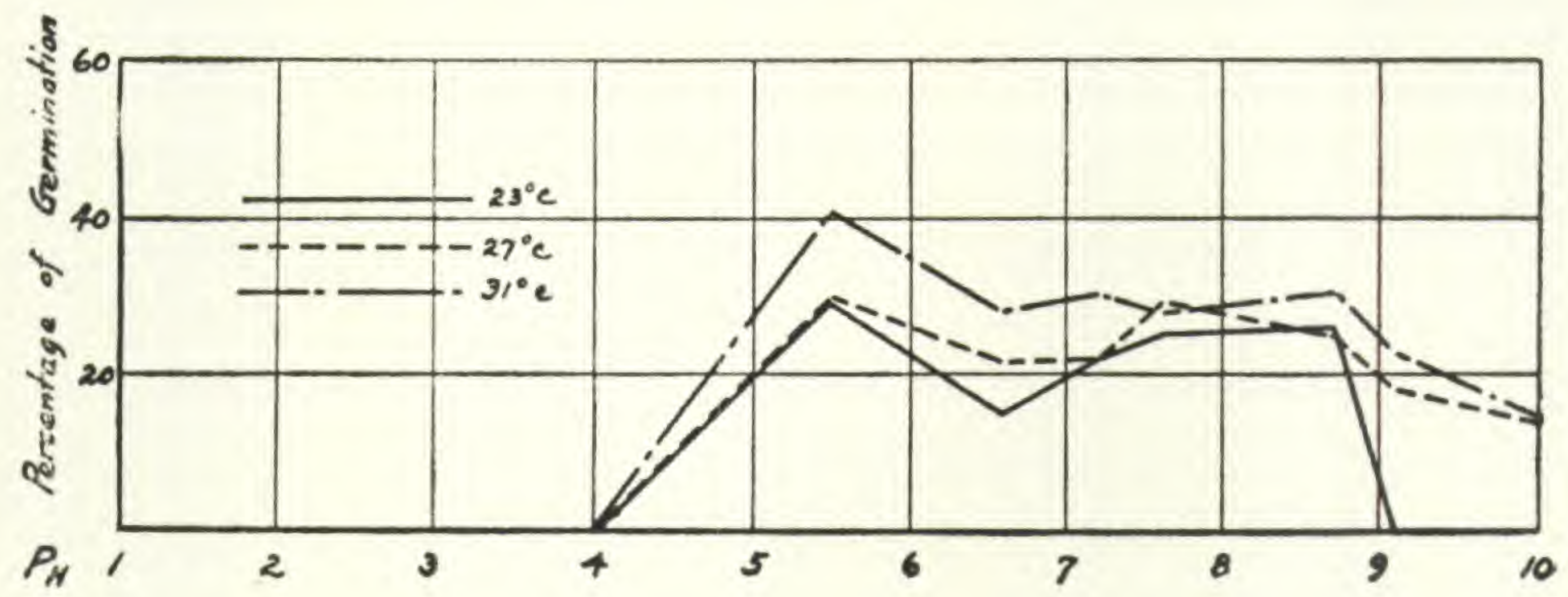

Fig. 36. *Colletotrichum Gossypii* in 2 per cent bacto-peptone solution.

in a broth medium between P_H 5.0 and 10.0, and that few spores ordinarily germinate at the H-ion concentrations closely approaching those of inhibition. In a few days, however, the spores germinate and exhibit all characteristic phenomena, thus indicating that by the life processes of the organism the reaction of the medium approaches that of the optimum. The final hydrogen-ion concentration of the medium, as determined from numerous other contributions for the bacteria, depend upon (1) the organism, (2) the composition of the medium, (3) the initial reaction, (4) the buffer effect, (5) the nature of the acid, (6) the period of incubation, and (7) other conditions favoring or hindering growth. It must be emphasized here that the P_H values which appear in this paper always represent, unless otherwise specified, the initial reaction or hydrogen-ion concentration of the medium.

The results of my experiments dealing with this phase of the problem do not seem to warrant an explanation entirely on the basis of change reaction of the medium during germination. The matter appears to be further complicated and obscured by other factors. *A. niger* produces no changes in solutions of mannite or in Czapek's solution, but does cause certain shifts towards neutrality in the alkaline cultures of the beet decoction. This organism produces in the presence of sugar an extremely large quantity of acid during growth, and it is not surprising that such reversions do occur in beet decoction, a solution poorly buffered and containing a high percentage of sugar. *B. cinerea* produces no changes in reaction while germinating in solutions of mannite, Czapek's solution, "water H_3PO_4 or NaOH" and "water HCl or KOH." However, it does produce changes in the beet decoction solutions similar to, but in less degree than

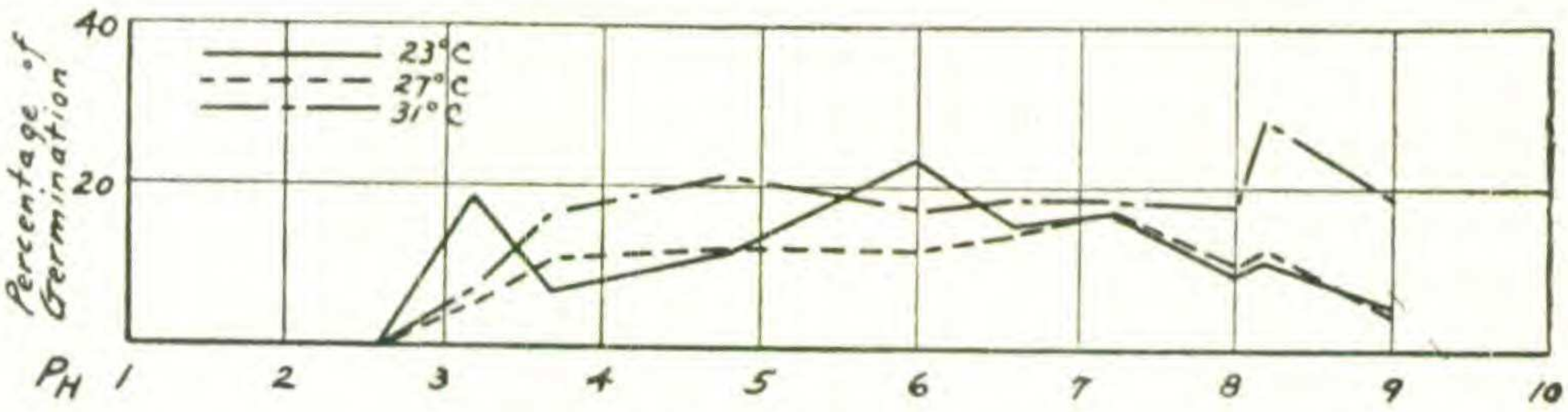

Fig. 37. *Colletotrichum Gossypii* in Czapek's full nutrient solution.

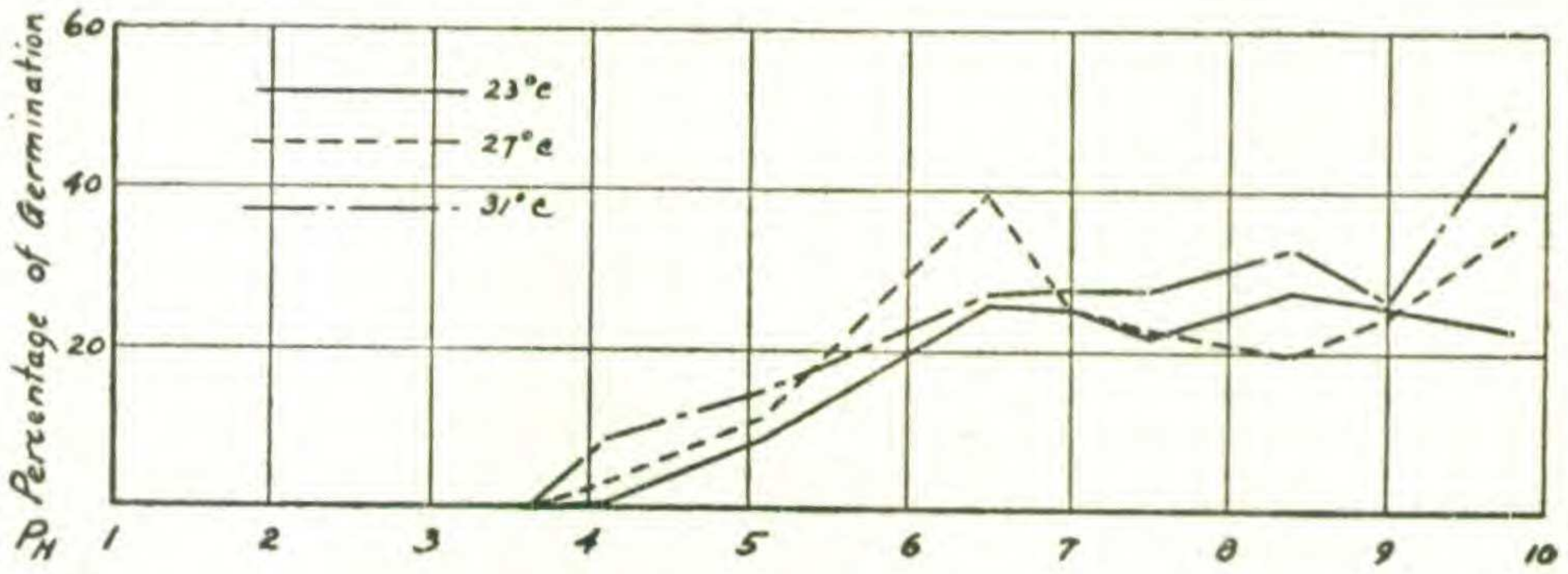

Fig. 38. *Colletotrichum Gossypii* in sugar beet decoction.

A. niger. The fact that no change whatsoever in reaction occurs with water lacking buffer effect and that relatively slight changes occur in beet decoction, a medium also poorly buffered, would seem to eliminate the possibility of explaining germination under conditions of active alkalinity entirely on the basis of change in reaction during this process. *Fusarium* sp. causes no changes in reaction of mannite and "water H_3PO_4 or NaOH" during germination. Slight changes are evidenced in the Czapek solutions, the more acid cultures shifting slightly towards neutrality. In "water HCl or KOH" all of the cultures possessing a

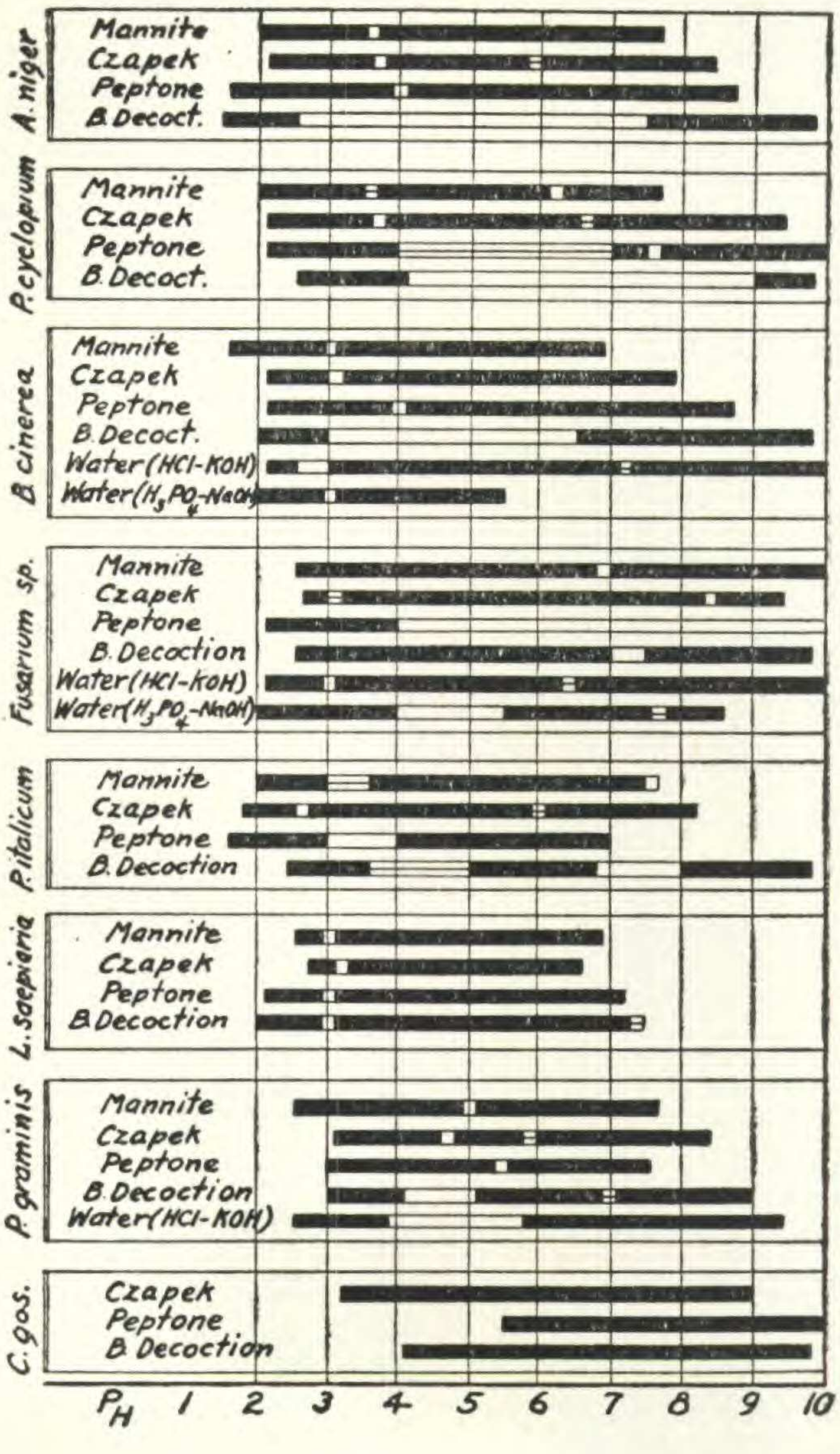

Fig. 39. Assembled data representing the averages obtained with the various temperatures employed and showing limits of germination (black), primary maximum (white), and secondary maximum (banded).

reaction favorable for germination exhibit some shift towards neutrality or towards slight alkalinity.

A temperature range of 4–5° C. on either side of a provisional optimum does not materially influence the germination of fungous spores as related to H-ion concentration. Of course, certain minor differences exist, but considering the great variability of spores, varying percentages of perfect germination, regardless of all precautions observed, will undoubtedly occur in any medium which is not a strong stimulus for germination. Germination at H-ion concentrations approaching more or less closely those causing inhibition is frequently irregular, and, in such cases the range of germination is usually widest at the provisional optimum.

The question of hydrogen-ion concentration relations will no doubt assist in the explanation of such problems in parasitism as host resistance, the establishment of strains of the parasite, etc.; and the varied capacity for germination of fungous spores may be found in time to be a suitable criterion for the isolation of strains. Nevertheless, the facts indicate that it is unwise to say that certain species of fungi or certain strains of fungi always germinate exclusively within a definite range of hydrogen-ion concentration. The limiting hydrogen-ion concentration of a fungus, as determined by germination, should be defined in terms of composition of the medium, and unless this is done—together with a consideration of other related factors—no classification of organisms can be made entirely upon any such basis.

SUMMARY

Using spores of the eight organisms, *Botrytis cinerea*, *Aspergillus niger*, *Penicillium cyclopium*, *P. italicum*, *Lenzites saepiaria*, *Puccinia graminis*, *Fusarium* sp., and *Colletotrichum Gossypii*, a comparative study has been made dealing with the effects of the hydrogen and hydroxyl-ion concentration upon germination in solutions of mannite, peptone, Czapek's full nutrient, sugar beet decoction, "water H_3PO_4 and NaOH" and "water HCl or KOH." Under the conditions described and as far as the experiments have gone, the following features of particular interest may be enumerated:

(1) Germination is a process which is strikingly supported by conditions of active acidity; relatively low percentages of germination, in most cases, are obtained under conditions of active alkalinity; and nutrition exerts a pronounced influence upon the relations of spore germination to the reaction of the medium.

(2) Successively increasing concentrations of hydrogen ions in the various culture solutions, from neutrality to approximately P_H 3.0 or 4.0, influence favorably the germination of the spores of *B. cinerea*, *A. niger*, *Penicillium cyclopium*, *P. italicum*, *Puccinia graminis*, *L. saepiaria*, and *Fusarium* sp. Moreover, *Fusarium* sp. germinates equally as well, if not better, with an alkaline reaction, and *C. Gossypii* is the only species which favorably responds only to an alkaline reaction.

(3) The majority of the fungi employed exhibit a distinct maximum of germination between P_H 3.0 and 4.0. Certain forms, notably *Fusarium* sp., exhibit secondary maximum at or near P_H 7.0.

(4) The range of germination and the magnitude of the germination quantities, as influenced by the hydrogen-ion concentration, depend upon both the organism and the medium.

(5) It is not until a hydrogen-ion concentration of P_H 1.5–2.5 is reached that inhibition of germination is evidenced. The limits on the acid side are comparatively narrow and constant. Those on the alkaline side are very diverse, varying with the organism and with the medium.

(6) Nutrition is an extremely important factor in the germination of fungous spores. In solutions of mannite, Czapek, and peptone, the relations of germination to hydrogen-ion concentration are more or less comparable for each particular organism; in beet decoction and "water HCl or KOH," on the other hand, results differing decidedly from those furnished by the previously mentioned solutions are obtained.

(7) Solutions of "water H_3PO_4 and NaOH," followed by those of mannite, undoubtedly give the smallest range of germination and the lowest percentages of germination. Solutions of bacto-peptone give very similar results to those developed in solutions of mannite, except that germination is better and is extended slightly more to the alkaline side. Czapek's full nutrient solution generally exhibits ranges similar to those furnished by the peptone solutions, but the curves of germination differ. In the Czapek solution, two maxima usually occur: a primary one between P_H 3.0 and 4.0 and a secondary one between P_H 6.0 and 7.0. Of all the media employed, beet decoction

gives the best germination and the widest range of germination. Germination in this medium, as well as in "water HCl or KOH," occurs freely under conditions of active alkalinity, producing inhibition of germination in the other media, and germination is stimulated to such an extent that the maxima cover relatively wide zones. The same may be said of the peptone medium, though the magnitude and the frequency are less. The other media, namely, mannite, Czapek's solution, and water, exhibit distinct maxima in the form of sharp peaks.

(8) Mycelial growth and development in the most acid and alkaline cultures permitting germination are relatively more feeble and scant, as determined visually, than in cultures possessing greater departures from the inhibiting concentrations. Rudimentary germ tubes or knob-like projections frequently occur in the most alkaline cultures, and abnormally and irregularly shaped germ tubes generally develop in the most acid cultures.

(9) Comparing equal concentrations of H and OH ions, the OH ions appear to be relatively more toxic to the spores studied than H ions. The toxicity of H ions is fairly independent of the other conditions studied, while that of the OH ions tends to be more or less variable or antagonizable, according to the composition of the medium.

(10) A change in reaction of the medium may or may not occur during germination, and, where shifts in the hydrogen-ion concentration do occur, the magnitude and nature of these depend upon the fungus, the medium, and the initial reaction. While growth stages are marked by pronounced changes in reaction of the medium, *A. niger* produces no changes in reaction while germinating in the solution of mannite and in Czapek's medium, but does produce certain shifts towards neutrality in the alkaline cultures of the beet decoction. *B. cinerea* induces no changes in the reaction of the medium while germinating in solutions of mannite, Czapek's solution, "water H_3PO_4 and NaOH," and "water HCl or KOH" but does produce a change in the beet decoction similar to but less than that of *A. niger*. The only conspicuous change brought about by germinating spores of *Fusarium* sp. is in "water HCl or KOH," all of the cultures giving favorable germination exhibiting a shift in reaction towards neutrality or slight alkalinity.

(11) The expanded range of germination under conditions of active alkalinity in such solutions as beet decoction and "water HCl or KOH" might appear on first glance to be correlated with buffer effects. Buffer action in these solutions is feebly present in the beet decoction and totally absent in the water. This is, no doubt, an important factor, but the experimental results here obtained seem hardly to justify an explanation entirely on such a basis. The fact that germinating spores of *B. cinerea* produce changes in reaction in the beet decoction medium and not in the "water HCl or KOH" is very striking.

(12) Various additions of sugar beet decoction to solutions of mannite pronouncedly stimulate germination with the two organisms under experimentation, namely, *A. niger* and *B. cinerea.* The percentages and the range of germination approach those offered by beet decoction alone, and no manifested resemblance to those furnished by the mannite alone is recognizable.

(13) It would seem, therefore, that germination in the beet decoction is stimulated by some special substance or peculiar set of conditions, either or both of which are totally or partially absent in the synthetic culture media.

(14) With increase in length of intervals of incubation, the relations of germination to hydrogen-ion concentration, other than a general increase in magnitude of all germination quantities and a frequent expansion of inhibitory limits, remain practically the same.

(15) The curves of germination for any organism are practically identical, whether incubated at a temperature representing a provisional optimum or at 4–5° C. above or below such an optimum. Germination often occurs feebly in cultures possessing a hydrogen-ion concentration closely approaching that of inhibition, and, if differences are manifested in the favorable ranges the tendency is for germination to occur over the widest range at the optimum temperature.

(16) The data here developed and presented, it is felt, are sufficient to change materially the previously prevailing view concerning the relation of germination of fungous spores to acid and alkaline media, as well as to be of fundamental importance in any future study of fungicides or spray mixtures.

The writer takes pleasure in acknowledging his indebtedness and extending his sincere thanks to Doctor B. M. Duggar for suggesting and directing the problem and for kindly criticism and advice throughout its prosecution; and to Doctor George T. Moore for the privileges and facilities of the Missouri Botanical Garden.

Graduate Laboratory, Missouri Botanical Garden.

Bibliography

Armstrong, G. M. ('21). Studies in the physiology of the fungi. XIV. Sulphur nutrition: the use of thiosulphate as affected by hydrogen-ion concentration. Ann. Mo. Bot. Gard. **8**: 237–281. *f. 1–21.* 1921.

Brooks, C. ('06). Temperature and toxic action. Bot. Gaz. **42**: 359–375. 1906.

Buller, A. H. R. ('06). The biology of Polyporus squamosus Huds., a timber destroying fungus. Jour. Econ. Biol. **1**: 101–138. *pl. 5–9. f. 1–51.* 1906.

Clark, J. F. ('99). On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. Bot. Gaz. **28**: 289–327, 378–404. 1899.

Clark, W. M., and Lubs, H. A. ('17). The colorimetric determination of hydrogen-ion concentration and its applications in bacteriology. Jour. Bact. **2**: 1–34, 109–136, 191–236. *f. 1–8.* 1917.

Cooley, J. S. ('14). A study of the physiological relations of Sclerotinia cinerea (Bon.) Schröter. Ann. Mo. Bot. Gard. **1**: 291–326. 1914.

Duggar, B. M. ('01). Physiological studies with reference to the germination of certain fungous spores. Bot. Gaz. **31**: 38–66. 1901.

————, ('09). Fungous diseases of plants. 1909. [See pp. 55–61.]

————, ('19). The micro-colorimeter in the indicator method of hydrogen-ion determination. Ann. Mo. Bot. Gard. **6**: 179–181. 1919.

————, and Dodge, C. W. ('19). The use of the colorimeter in the indicator method of H-ion determination with biological fluids. *Ibid.* **6**: 61–70. *f. 1.* 1919.

————, Severy, J. W., and Schmitz, H. ('17). Studies in the physiology of the fungi. IV. The growth of certain fungi in plant decoctions. *Ibid.* **4**: 165–173. *f. 1–4.* 1917.

Falck, R. ('12). Hausschwamforschungen. Zeitschr. f. Forst. u. Jagdwesen. 6: 1–405. *pl. 1–17.* 1912. [See pp. 273–280.]

Ferguson, Margaret C. ('02). A preliminary study of the germination of the spores of Agaricus campestris and other basidiomycetous fungi. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 16: 1–40. *pl. 1–3.* 1902.

Gillespie, L. J. ('18). The growth of the potato scab organism at various hydrogen-ion concentrations as related to the comparative freedom of acid soils from the potato scab. Phytopath. **8**: 257–269. *f. 1.* 1918.

Hopkins, E. F. ('21). Growth and germination of Gibberella saubinetti at varying hydrogen-ion concentrations. [*Ibid.* **11**: 36. 1921.]

Itano, A., and Neil, J. ('19). Influence of temperature and hydrogen-ion concentration upon the spore cycle of Bacillus subtilis. Jour. Gen. Physiol. **1**: 421–428. *f. 1–2.* 1919.

Karrer, Joanne L. ('21). Studies in the physiology of the fungi. XII. The effect of hydrogen-ion concentration upon the accumulation and activation of amylase produced by certain parasitic fungi. Ann. Mo. Bot. Gard. **8**: 63–96. *f. 1–14.* 1921.

————, and Webb, R. W. ('20). Titration curves of certain liquid culture media. *Ibid.* **7**: 299–305. *f. 1–5.* 1920.

Krönig, B., and Paul, T. ('97). Die chemischen Grundlagen der Lehre von der Giftwirkung und Desinfektion. Zeitschr. f. Hyg. u. Infekt. **25**: 1–112. *pl. 1.* 1897.

Meacham, M. R. ('18). Note upon the hydrogen ion concentration necessary to inhibit the growth of four wood destroying fungi. Science N. S. **48**: 499–500. *f. 1.* 1918.

Peltier, G. L. ('12). A consideration of the physiology and life history of a parasitic Botrytis on pepper and lettuce. Mo. Bot. Gard., Ann. Rept. **23**: 41–74. *pl. 1–5.* 1912.

Spaulding, P. ('11). The timber rot caused by Lenzites saepiaria. U. S. Dept. Agr., Bur. Pl. Ind. Bul. 214: 1–46. *pl. 1–4.* 1911.

Stakman, E. C. ('13). Spore germination of cereal smuts. Minn. Agr. Exp. Sta., Tech. Bul. 133: 1–77. *pl. 1–25.* 1913.

Steinberg, R. A. ('19). A study of some factors in the chemical stimulation of the growth of Aspergillus niger. Am. Jour. Bot. **6**: 330–372. *f. 1–2.* 1919.

Stevens, F. L. ('98). The effect of aqueous solutions upon the germination of fungous spores. Bot. Gaz. **26**: 377–406. 1898.

Stoneman, B. ('98). A comparative study of the development of some anthracnoses. *Ibid.* **26**: 69–120. *pl. 7–18.* 1898.

Thiel, A. F., and Weiss, F. ('20). The effect of citric acid on the germination of the teliospores of Puccinia graminis. Phytopath. **10**: 448–452. *f. 1.* 1920.

Taylor, K. ('17). Specificity of antiseptics. The Lancet **1**: 294–297. 1917.

Webb, R. W. ('19). Studies in the physiology of the fungi. X. Germination of the spores of certain fungi in relation to hydrogen-ion concentration. Ann. Mo. Bot. Gard. **6**: 201–222. *f. 1–5.* 1919.

Wright, J. H. ('17). The importance of uniform culture media in the bacteriological examination of disinfectants. Jour. Bact. **2**: 315–346. 1917.

Zeller, S. M. ('16). Studies in the physiology of the fungi. II. Lenzites saepiaria Fries, with special reference to enzyme activity. Ann. Mo. Bot. Gard. **3**: 439–512. *pl. 8–9.* 1916.

————, ('20). Humidity in relation to moisture imbibition by wood and to spore germination on wood. *Ibid.* **7**: 51–74. *pl. 1. f. 1–5.* 1920.

————, Schmitz, H., and Duggar, B. M. ('19) Studies in the physiology of the fungi. VII. Growth of wood-destroying fungi on liquid media. *Ibid.* **6**: 137–142. 1919.